DOSE-DEPENDENT EFFECT OF RETINOL ON CLINICAL AND ORGAN PROFILES IN PREGNANT AND NON-PREGNANT WISTAR RATS.

BY

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A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE, FACULTY OF BASIC MEDICAL AND HEALTH SCIENCES, THOMAS ADEWUMI UNIVERSITY, OKO, KWARA STATE, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF MEDICAL LABORATORY SCIENCE (B.MLS)

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ABSTRACT

Retinol (vitamin A) is an essential micronutrient, but excessive intake, particularly during pregnancy, can be toxic. This study evaluated the dose-dependent effects of retinol on haematological and liver health in pregnant and non-pregnant Wistar rats. Thirty-two female Wistar rats were divided into pregnant and non-pregnant groups (n=4), receiving control, minimal, therapeutic, or lethal doses of retinol for 21 days. The blood samples were analyzed for haematology, liver function and liver histopathology. Minimal and therapeutic doses did not significantly alter most haematological or liver function parameters. However, pregnant rats showed a dose-dependent increase in haemoglobin, suggesting enhanced erythropoiesis. Lethal doses increased liver weight and caused severe hepatocellular degeneration, necrosis, and cholestasis, effects that were more pronounced in pregnant animals. Despite this, liver function tests were within normal ranges, indicating subclinical damage. Retinol's effects are dose-dependent, with high doses inducing hepatotoxicity and haematological changes, especially during pregnancy, highlighting the need for caution with vitamin A supplementation during gestation.

Key words: Retinol, Pregnancy, Hepatotoxicity, Haematology, Dose-dependent effects and Wistar rats

DECLARATION

I, SULEMAN FARIDAT REJOICE hereby declare that this research work “Dose-dependent effect of retinol on clinical and organ profiles in pregnant and non-pregnant wistar rat” is my original project work and has not been previously submitted elsewhere or in this University for the award of a degree.

06-08-2025

Suleman Faridat Rejoice (Date)

CERTIFICATION PAGE

I declare that this project is my original work and has not been previously submitted to any other institution of higher learning.

I further certify that all sources cited or quoted are duly acknowledged by means of comprehensive list of references.

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DEDICATION

This project is whole heartedly and specially dedicated to God who by His mercy saw me through to the end of this project despite all odds.

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LIST OF ABBREVIATIONS

ROL – Retinol

RE – Retinyl Ester

RA – Retinoic Acid

atRA – All-trans Retinoic Acid

9cRA – 9-cis Retinoic Acid

RBP – Retinol-Binding Protein

STRA6 – Stimulated by Retinoic Acid 6

TTR – Transthyretin

CRBP – Cellular Retinol-Binding Protein

CRABP – Cellular Retinoic Acid-Binding Protein

LRAT – Lecithin Retinol Acyltransferase

REH – Retinyl Ester Hydrolase

ALDH1A1 – Aldehyde Dehydrogenase 1 Family Member A1

CYP26A1 – Cytochrome P450 Family 26 Subfamily A Member 1

CYP26B1 – Cytochrome P450 Family 26 Subfamily B Member 1

Gene Regulation & Retinoid Receptors

RAR – Retinoic Acid Receptor

RXR – Retinoid X Receptor

RARE – Retinoic Acid Response Element

RBC – Red Blood Cell

WBC – White Blood Cell

Hb – Hemoglobin

HCT – Hematocrit

MCV – Mean Corpuscular Volume

MCH – Mean Corpuscular Hemoglobin

MCHC – Mean Corpuscular Hemoglobin

PLT – Platelet

H&E – Hematoxylin and Eosin

FGR – Fetal Growth Restriction

TGF-β – Transforming Growth Factor Beta

Treg – Regulatory T Cell

Th – T Helper Cell

DC – Dendritic Cell

IL – Interleukin (e.g., IL-2, IL-10)

IFN-γ – Interferon Gamma

TNF-α – Tumor Necrosis Factor Alpha

EFSA – European Food Safety Authority

FDA – Food and Drug Administration

WHO – World Health Organization

CNS – Central Nervous System

ROS – Reactive Oxygen Species

CHAPTER ONE

INTRODUCTION

1.0 Background of Study

Vitamin A (retinol) is a fat-soluble micronutrient essential for multiple physiological functions, including vision, immune competence, cellular differentiation, reproduction, and hematopoiesis (Hodge and Taylor, 2023). Its importance becomes particularly pronounced during pregnancy, a complex physiological state involving extensive metabolic, haematological, and immunological adaptations to support fetal development and maternal health. The increased demand for micronutrients during this period makes the regulation of vitamin A intake critical, as both deficiency and excess can pose significant health risks. Dietary vitamin A is available in two major forms: preformed retinol from animal-based sources and provitamin A carotenoids from plant-derived foods. Upon ingestion, these are metabolized into active compounds such as retinoic acid, which functions as a regulatory molecule influencing gene expression, tissue development, and immune responses (Patili *et al.,* 2023). During gestation, retinol contributes to organogenesis, hematopoiesis, and immune modulation. However, vitamin A deficiency in pregnant women prevalent in many low- and middle-income countries has been linked to anemia, increased susceptibility to infections, and adverse pregnancy outcomes (Onigemo *et al.,* 2020). Conversely, hypervitaminosis A, often resulting from supplementation or excessive intake of synthetic derivatives, can lead to hepatotoxicity, renal impairment, teratogenic effects, and biochemical disturbances (Patili *et al.,* 2023). Animal models such as Wistar rats (*Rattus norvegicus*) are widely employed in nutritional and toxicological research due to their well-characterized physiology (Mukherjee et al.,

2022), controlled genetic backgrounds, and translational relevance to human health (Hulme-Beaman *et al.,* 2021; Patel *et al.,* 2024). These models offer a robust platform for examining the dose-dependent effects of micronutrients like retinol on vital organ systems and metabolic processes. This study explores the impact of varying levels of retinol intake on haematological, biochemical (chemical pathology), and histopathological parameters in both pregnant and non-pregnant Wistar rats. haematological indices including red and white blood cell counts, hemoglobin concentration, and platelet levels serve as vital indicators of oxygen transport, immune competence, and hematopoietic activity. Alterations in these markers may signify underlying nutritional imbalances or toxic effects. Biochemical assessments focus on liver and kidney function markers to evaluate organ health, enzyme activity, and metabolic efficiency, while histopathological examination of hepatic and renal tissues provides insight into cellular architecture and potential pathological changes such as degeneration, necrosis, inflammation, or vacuolation. By integrating these multidisciplinary assessments, the study aims to establish a clearer understanding of how retinol influences systemic physiology, particularly under the altered metabolic conditions of pregnancy. Ultimately, the findings will inform evidence-based recommendations for safe vitamin A supplementation, reduce risks associated with deficiency or toxicity, and enhance maternal and fetal health outcomes.

1.1 Statement of Problem

Pregnancy being a critical phase in a woman's life is marked by significant physiological and metabolic changes that profoundly influence both maternal and fatal health. Among the essential nutrients required for optimal pregnancy outcomes, retinol (vitamin A) plays a vital role not only in cellular differentiation, immune

system function, and fetal development, but also in maintaining epithelial integrity, supporting hematopoiesis, and regulating gene expression critical for maternal and embryonic health (Maia *et al.,* 2019) but despite its importance, the precise effects of varying retinol levels on hematological parameters and histological changes in pregnant populations are not well understood. Existing research highlights the dual risks associated with retinol: deficiency can lead to immune dysfunction, vision problems, and increased susceptibility to infections, while excessive intake may result in toxicity, impairing liver function and disrupting normal physiological processes (Li *et al.,* 2021). Pregnant females experience heightened nutritional demands, rendering them particularly susceptible to both deficiency and excess of critical micronutrients such as retinol. However, existing literature presents inconsistent and inconclusive findings regarding the optimal levels of retinol intake during pregnancy, thereby complicating the understanding of its impact on key hematological and biochemical parameters, as well as its influence on histological alterations in vital maternal and fetal organs. This lack of clarity hinders the development of Evidence Based guidelines for vitamin A intake during pregnancy, leaving healthcare providers without definitive recommendations (Olsen *et al.,* 2023). It has been seen that this inconsistency in existing studies, highlights the need for standardized guidelines (Shastak and Pelletier, 2023).

Furthermore, the potential risks of hypervitaminosis A in pregnant populations highlight the necessity for a thorough investigation into the relationship between retinol intake and its physiological effects (Carazo *et al.,* 2021). Consequently, this study aimed to rigorously evaluate the impact of varying retinol dosage levels on haematological parameters and histological changes in pregnant Wistar rats. The goal is to provide essential insights for developing evidence-based guidelines on safe

retinol supplementation during pregnancy. By using pregnant Wistar rats as an animal model, this research sought to address existing knowledge gaps and elucidate the optimal balance of vitamin A needed for maternal and fetal health, ultimately guiding dietary recommendations to ensure the well-being of both mothers and their developing offspring (Ishaq *et al.,* 2023).

1.3 Study Justification

The necessity of this study is underscored by several critical factors that highlight both its scientific relevance and potential public health impact. Firstly, there exists a substantial knowledge gap in the current literature regarding optimal retinol intake during pregnancy. While vitamin A is recognized as essential for maternal and fetal health, existing studies report inconsistent findings and a lack of consensus on safe and effective supplementation thresholds. These discrepancies complicate clinical recommendations and raise concerns over both under-supplementation and potential toxicity (McCauley *et al.,* 2015). Secondly, the clinical significance of retinol is well-established. Both deficiency and excessive intake are associated with serious maternal and fetal complications, including congenital malformations, spontaneous abortions, preterm birth, and increased maternal morbidity and mortality. These adverse outcomes underscore the need for a more balanced and evidence-based approach to vitamin A supplementation in pregnancy (Ishaq *et al.,* 2024).

Moreover, the selection of pregnant Wistar rats as the model organism is justified by their well-characterized reproductive physiology, which bears considerable resemblance to that of humans. This similarity enables the generation of experimentally controlled data that are relevant and translatable to human maternal health. Wistar rats are widely used in nutritional, hematological, and toxicological

research, reinforcing the validity of using them to investigate the physiological consequences of varying retinol levels. Importantly, while much of the existing literature focuses primarily on the fetal developmental effects of vitamin A, there remains a significant research gap concerning its influence on maternal hematological parameters, biochemical indices, and histological changes. This study aims to address that void by evaluating how different levels of retinol affect maternal blood profiles, organ function, and tissue integrity especially in the liver and kidney, which are central to vitamin A metabolism and detoxification (Sombie *et al.,* 2023). The potential public health implications of this research are considerable. By deepening our understanding of how retinol influences maternal physiological systems, the findings can contribute to the development of evidence-based nutritional guidelines aimed at reducing the risks associated with vitamin A imbalance during pregnancy. Ultimately, this study seeks to inform clinical practices, support preventive health strategies, and promote healthier pregnancy outcomes in both high-risk and general populations.

1.4 Research Aim and Objectives

1.4.1 Aim

To investigate the effects of varying doses of retinol on haematological, biochemical and histopathological parameters in pregnant and non-pregnant Wister rats.

Objectives

The specific objectives are to;

I. To evaluate the effect of different doses of retinol on haematological parameters in both pregnant and non-pregnant Wistar rats.

II. To determine the impact of varying doses of retinol on biochemical parameters, pregnant and non-pregnant Wistar rats.

III. To examine the histopathological alterations in the liver associated with retinol administration across different dosage levels in both pregnant and non-pregnant Wistar rats.

1.4 Research Questions

I. What are the effects of different doses of retinol intake on haematological parameters in pregnant and Non-pregnant Wistar rats?

II. What are the effects of different doses of retinol intake on biochemical parameters in pregnant and Non-pregnant Wistar rats?

III. What are the effects of different doses of retinol intake on histopathological parameters in pregnant and Non-pregnant Wistar rats?

1.5 Research Hypothesis

I. Null Hypothesis (H_0): Administration of varying doses of retinol has no significant effect on liver function, haematological parameters, or liver histology in pregnant and non-pregnant Wistar rats.

II. Alternative Hypothesis (H_1): Administration of varying doses of retinol significantly affects liver function, haematological parameters, and liver histology in pregnant and non-pregnant Wistar rats.

1.6 Significance of the Study

This study is to enhance our understanding of retinol's impact on clinical laboratory parameters and histological changes in pregnant and non-pregnant Wistar rats, offering valuable insights into maternal and fetal physiology. The findings will support research-backed recommendations for vitamin A intake in both pregnancy and non-pregnancy contexts, addressing the need for precise nutritional guidelines to safeguard maternal, fetal, and general health. By clarifying retinol's physiological roles and its safe intake levels, the study aims to inform healthcare providers and nutrition policymakers about how to prevent both deficiency and toxicity. This is especially important for vulnerable populations at risk of vitamin A imbalances.

Additionally, the research will help identify adverse effects linked to hypervitaminosis A, thereby promoting safer consumption practices. On a practical level, the study has the potential to improve maternal nutrition, reduce pregnancy-related complications, and enhance birth outcomes. It will also aid in the development of strategies that integrate retinol within broader maternal health initiatives, contributing to safer pregnancies and healthier deliveries. Theoretically, this research will expand the scientific understanding of retinol's mechanisms of action and its interaction with other micronutrients. It may inspire new therapeutic approaches incorporating vitamin A to optimize maternal and fetal well-being. Societally, these findings can shape public health policies, improve clinical practices, reduce retinol-related health burdens, and ultimately promote healthier populations through more effective resource allocation.

1.7 Sustainable Development Goals (SDGs)

The methodology adopted in this study aligns with several Sustainable Development Goals (SDGs) by maintaining scientific integrity, ethical research practices, and direct relevance to maternal and child health. It supports SDG 3 (Good Health and Well-being) by examining how retinol affects fetal development, immune function, and hematopoiesis. Retinoic acid is essential for embryonic hematopoietic stem cell differentiation and immune regulation (Purton *et al.,* 2007; Cano *et al.,* 2014). This study also highlights the risks of both vitamin A deficiency and toxicity during pregnancy, contributing to efforts to reduce maternal and infant morbidity and mortality. Aligned with SDG 2 (Zero Hunger), the research underscores vitamin A's critical role in maternal nutrition and aims to inform dietary recommendations in regions where deficiency remains prevalent (Czuba *et al.*, 2022). Furthermore, in support of SDG 10 (Reduced Inequalities), the study considers disparities in vitamin A access and the health consequences among underserved populations. By adhering to ethical animal research protocols, the study contributes to SDG 12 (Responsible Consumption and Production), ensuring efficient and responsible use of research resources. It also promotes SDG 17 (Partnerships for the Goals) by generating findings that can inform global nutrition policies and encourage collaboration among public health stakeholders.

1.8 Scope of the Research

The scope of this research investigates the dose-dependent effects of retinol intake on selected hematological, biochemical, and histological parameters in both pregnant and non-pregnant Wistar rats. The animal model is chosen for its physiological and reproductive similarities to humans, making it suitable for exploring maternal-fetal

responses to micronutrient modulation. The study will involve four groups receiving different levels of retinol: a control group (normal diet), a minimal-dose group, a therapeutic-dose group, and a high-dose (lethal) group.

Key hematological indices including total white blood cell count (TWBC) with differentials, and platelet (PLT) count will be evaluated to assess the impact of retinol on oxygen transport, immune competence, and hemostatic balance. In parallel, biochemical parameters reflecting liver function such as serum alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), albumin, total protein, urea, and creatinine was measured to detect subclinical or overt organ dysfunction resulting from either deficiency or toxicity.

Histological examinations of the liver was conducted to observe cellular and structural changes, including hepatocellular degeneration, sinusoidal dilation, inflammatory infiltration, tubular necrosis, or glomerular alterations, which may arise due to excessive or insufficient retinol exposure. These morphological assessments was to support the biochemical data and provide visual confirmation of organ integrity or injury. Incorporating both pregnant and non-pregnant rats ensures a broader understanding of how gestational status influences susceptibility to retinol-associated alterations.

The study also considers how varying doses affect maternal health and the development of the fetus, thereby offering insight into pregnancy-specific nutritional needs and toxicological risks. By integrating hematological, biochemical, and histopathological evaluations across graded retinol doses, this study aims to generate evidence that informs safe vitamin A intake guidelines, particularly during pregnancy. The findings are to contribute to existing literature on micronutrient safety and

toxicity, while also guiding future research on retinoid interactions with other nutrients during gestation.

CHAPTER TWO

LITERATURE REVIEW

2.0 Introduction

Understanding the biological significance, mechanisms, and health implications of vitamin A (retinol) requires a structured review of existing literature to contextualize its role in human and animal physiology particularly during pregnancy. This chapter presents a comprehensive literature review organized into three major sections: Conceptual Review, Theoretical Review, and Empirical Review. This tripartite structure allows for a coherent exploration of retinol from foundational concepts to theoretical frameworks and Scientifically supported protocol.

Vitamin A and its active metabolite, retinoic acid, which is synthesized from retinol, are vital for numerous physiological processes in both embryonic development and adult life (Shannon *et al*., 2020). They are critical for normal growth, regulation of epithelial tissue, proliferation, differentiation, support of visual and reproductive functions (McEldrew *et al*., 2023). Retinoids, which is a derivative of vitamin A, have become indispensable in the treatment of dermatological disorders, including acne, psoriasis, ichthyosis, and in oncology applications (Ramchatesingh *et al*., 2022). Because animals cannot synthesize vitamin A endogenously, it must be obtained from dietary sources, such as carotenoids from plants or retinyl esters from animal products (Carazo *et al*., 2021). Vitamin A is distinguished by its unique unsaturated isoprenoid chain structure, which is essential for its roles in vision, immune function, and cellular differentiation (Lerner, 2024). It exists in various forms, including retinol, retinyl esters, retinaldehyde, and all-trans-retinoic acid (tretinoin), all of which share a similar molecular framework and contribute to these biological functions (Motamedi *et al*., 2021). Collectively, these compounds belong to a broad class of molecules

known as retinoids, encompassing both naturally occurring forms of vitamin A and synthetic analogs (Quan, 2023).

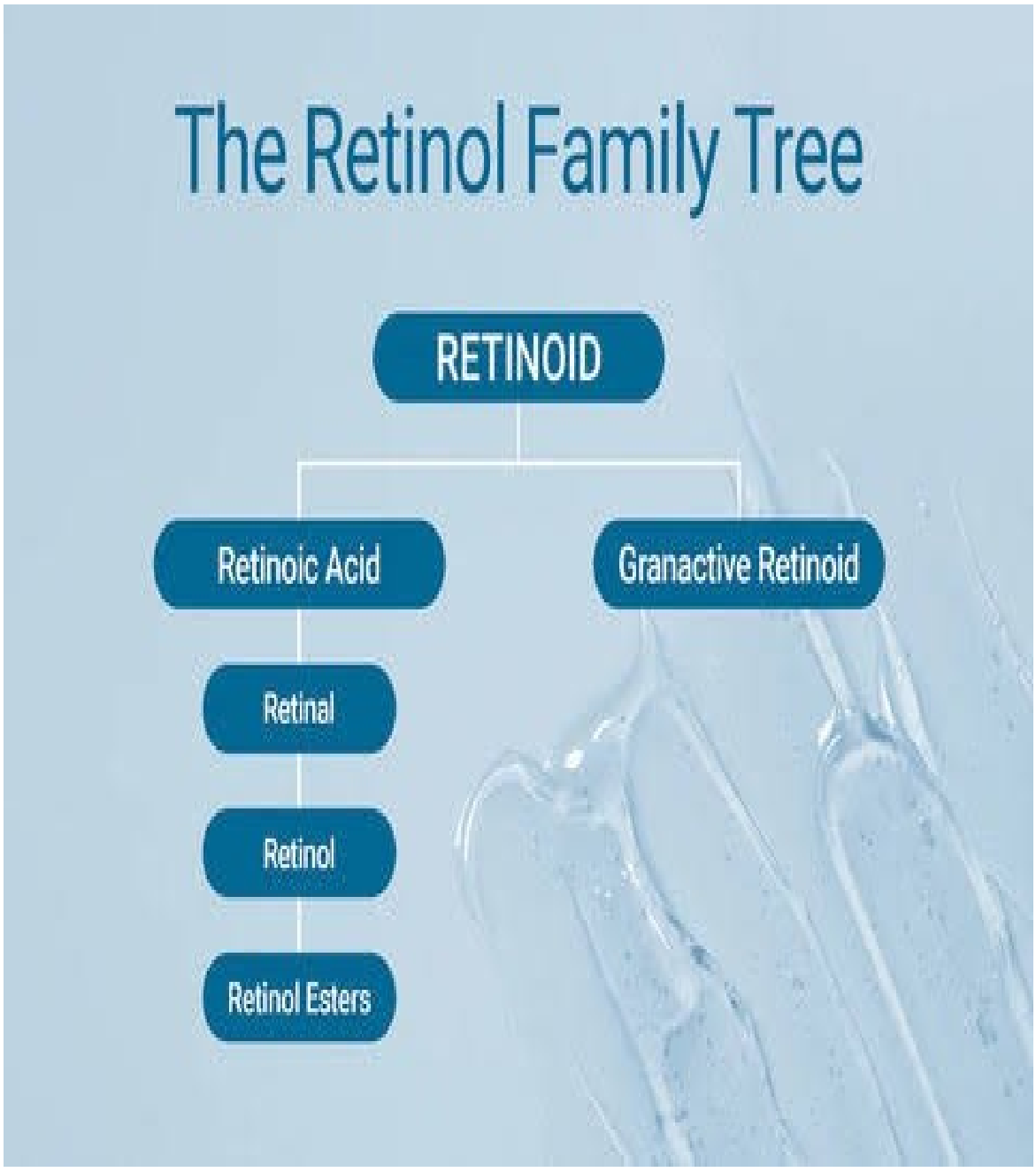

Figure 2.1: The Retinol (101) Family Tree (Neutriherbs Editors, 2023)

In vertebrates, vitamin A is stored primarily in the liver as retinyl esters, with additional reserves found in the lungs, kidneys, and bone marrow (Carazo *et al*., 2021). This storage mechanism acts as a buffer during periods of dietary deficiency, ensuring a constant supply of vitamin A (D'Ambrosio *et al*., 2011; O'Connor *et al.,* 2022). Retinol, the transportable form of vitamin A, circulates in the bloodstream and then bound to retinol-binding protein (RBP), which protects it from degradation and facilitates its delivery to cells that require retinoic acid for key metabolic functions (Knight *et al*., 2024). Upon binding to the RBP receptor STRA6, retinol is internalized into cells, where it is converted reversibly to retinal by retinol dehydrogenases (RODHs or ADHs). Retinal is then irreversibly oxidized to retinoic acid by retinal dehydrogenases (RALDHs), a process critical for retinoic acid's role in gene regulation (Cho *et al*., 2021). Once inside the cell, retinoic acid binds to cellular retinoic acid-binding proteins (CRABPs), ensuring its proper transport and function in the cytoplasm (Napoli, 2016).

Retinoids, both natural and synthetic derivatives of vitamin A, share a core structure composed of four isoprenoid units. Despite structural variations between synthetic and natural retinoids, they retain functional similarities due to their common vitamin A structure (Kawczak *et al.*, 2024), which allows them to interact with retinoid receptors and contribute to biological functions, such as skin differentiation and immune regulation (Redfern, 2020). Vitamin A's lipophilic properties enable its accumulation in tissues like the liver and adipose tissue (Blaner, 2020). This accumulation provides a critical reserve during periods of dietary deficiency, but excessive intake can lead to toxicity (Olson *et al.*, 2023). Dietary sources of vitamin A are predominantly animal-based, including liver and dairy products, along with plant-derived provitamin A carotenoids that the body can convert into active vitamin A. The

biological activity of vitamin A and its analogs is measured using a unit system. One international unit (IU) of vitamin A is equivalent to 0.3 mcg of all-trans-retinol, and retinol equivalents (RE) standardize different sources. For example, 1 mcg of retinol is biologically equivalent to 6 mcg of beta-carotene or 12 mcg of mixed dietary carotenoids, with 25,000 IU equating to 7.5 mg of all-trans-retinol. (Teratology Society, 1987; Ross, 2024)

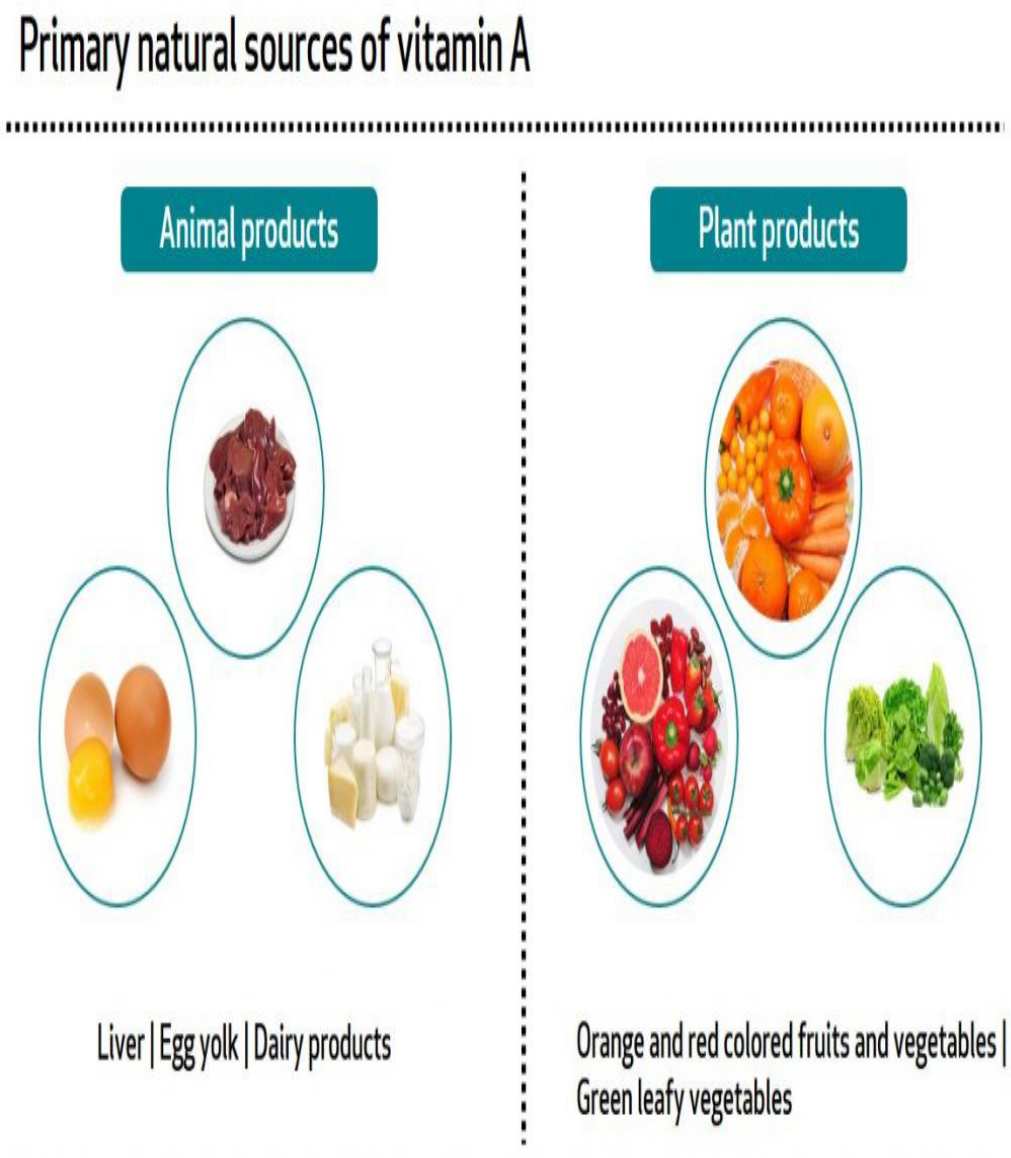

Figure 2.2: Primary natural animal and plant sources of vitamin A (Bioanalyt, 2020).

Although retinol is commonly associated with vitamin A, its active forms in the body 11-cis-retinal and all-trans-retinoic acid (ATRA) are crucial for vision and cellular regulation, respectively Bohn *et al*., 2019). Carotenoids are groups of naturally occurring pigments that are responsible for the red, orange, and yellow colors seen in fruits, vegetables, eggs, meats, milk, and some seafood. There are over 700 carotenoids found in nature, but only around 50 are present in the human diet, with about half of these detectable in human blood and tissues, as outlined in the review by Krinsky and Johnson. The primary carotenoids in human serum include β-carotene, α-carotene, lycopene, lutein, zeaxanthin, and β-cryptoxanthin and some carotenoids also contribute to vitamin A metabolism through their antioxidant properties (Gebregziabher *et al.,* 2023), which is essential for functions such as vision, epithelial cell regeneration, and regulating gene expression through retinoic acid, (a vitamin A metabolite), as reviewed by Tanumihardjo *et al*. Certain carotenoids, such as β-carotene, can be converted into retinol, but only those with an unsubstituted β-ionone ring exhibit provitamin A activity (Harrison, 2022). Epidemiological studies suggest that carotenoids may also play a role in reducing the risk of chronic diseases like cancer, cardiovascular diseases, and age-related macular degeneration, while improving cognitive and visual functions, However, clinical trials are necessary to establish a causal relationship between carotenoid intake and these health benefits. The regulation of retinoic acid synthesis and signaling is essential for various physiological functions, and its dysregulation can lead to conditions such as night blindness, developmental defects, and immune dysfunction. Understanding these pathways also holds therapeutic potential for conditions such as cancer and in regenerative medicine, emphasizing the importance of continued research into retinoic acid metabolism and vitamin A function (Connolly *et al*., 2013; Lavudi *et al*.,2020).

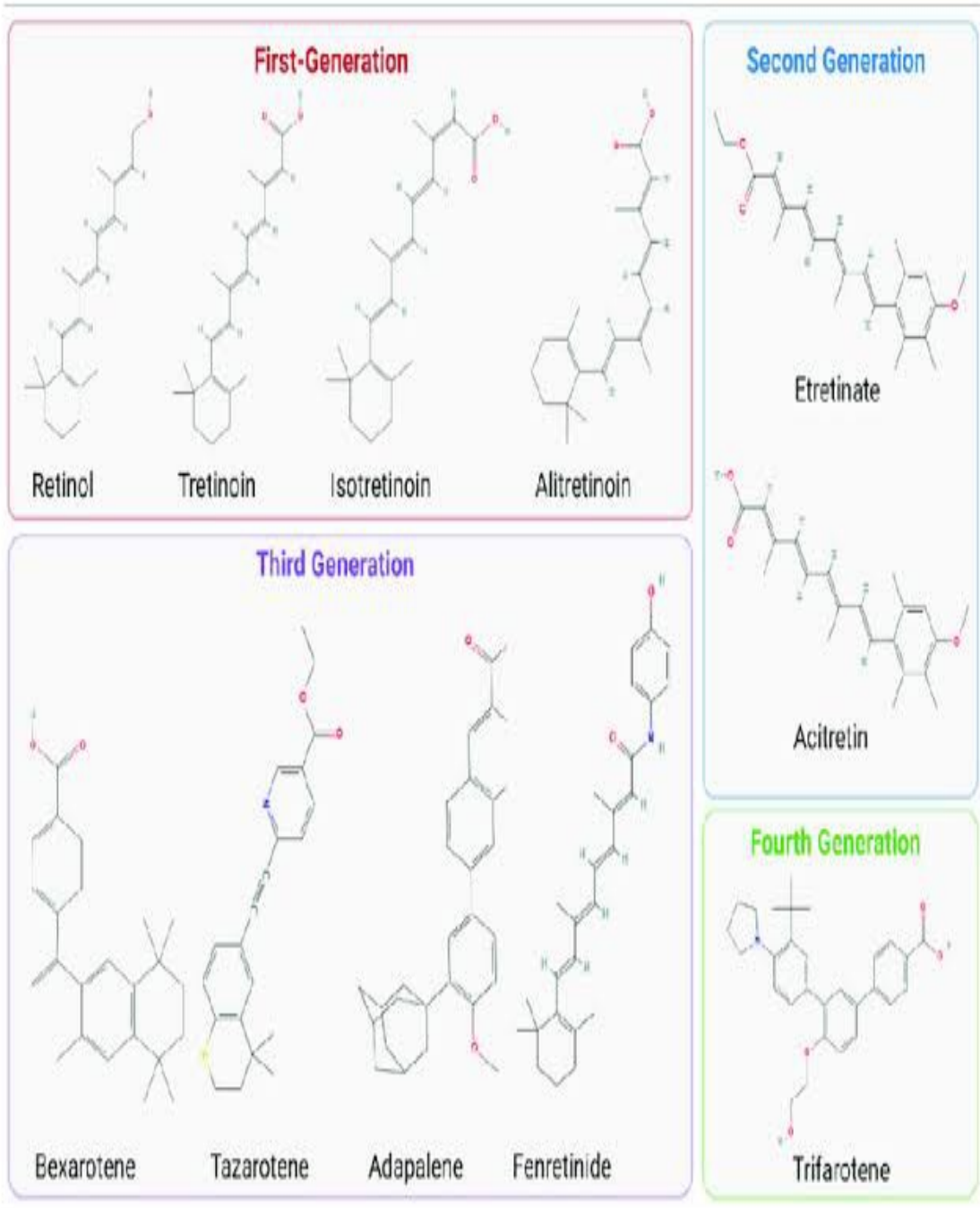

Figure 2.3: Chemical structures of clinically used retinoids from each generation. Adapted from PubChem (2022).

The wide-ranging physiological roles of retinoids have prompted the development of a variety of synthetic derivatives, classified into four generations. First-generation (non-aromatic) retinoids include naturally occurring compounds such as retinol, retinal, all-trans-retinoic acid (ATRA), 9-cis-retinoic acid (alitretinoin), and 13-cis-retinoic acid (isotretinoin) (Kawczak *et al.,* 2024). Second-generation (mono-aromatic) retinoids are synthetic agents like etretinate and acitretin (Zito *et al*., 2024), while third-generation (poly-aromatic) retinoids include compounds like adapalene, tazarotene, and bexarotene (Tolaymat *et al.,* 2023). The most recent, fourth-generation retinoid, trifarotene (Naik, 2022), has been approved for clinical use in the United States. Various synthetic retinoids have been developed to retain the therapeutic benefits of retinoids while minimizing side effects commonly associated with natural forms (Office of Dietary Supplements, 2024). The rising use of vitamin A and its analogs, particularly in developed nations like the United States, reflects a broader trend of excessive nutrient intake, often through high-dose supplements (Olson *et al.,* 2023). Figures such as Linus Pauling (1986) and Adele Davis (1970) have popularized this trend, but concerns over the unregulated use of vitamin A, especially during pregnancy, have emerged. Excessive intake during pregnancy can increase the risk of congenital abnormalities, as the teratogenic effects of vitamin A derivatives are well-documented in animal models (Abadie *et al.,* 2023). Clinical reports of fetal malformations in children born to mothers who consumed high doses of vitamin A during pregnancy emphasize the need for caution when using Vitamin A during this critical period (Maia *et al.,* 2019). Retinol and its derivatives are integral to critical biological processes, including vision, immune function, cellular growth, differentiation, and metabolism (McEldrew, 2022; Kawczak*et al.,* 2024). Retinoids are also crucial for gene regulation, essential for development and tissue maintenance.

During pregnancy, various hematological parameters are significantly altered, especially during the early and mid-stages (Chandra *et al.,* 2012).

2.1 Structure of Retinol and Its Biological Significance

Retinol (C20H30O) is a lipid alcohol distinguished by its complex molecular architecture, which is fundamental to its various biological roles (National Centre for Biotechnology Information, 2023). Its structure encompasses three primary components: a β-ionone ring, a long hydrophobic carbon chain, and a terminal hydroxyl group (Knight *et al.,* 2024). This intricate configuration is crucial for retinol's function as a ligand for nuclear retinoid receptors, influencing gene expression and playing significant roles in hematological parameters, histological changes, and interactions with serum proteins (Steinhoff *et al.,* 2022), including retinol-binding protein (RBP).

The β-ionone ring serves as a chromophore, enabling light absorption critical for vision (Corbo, 2021). The hydrophobic carbon chain enhances retinol's lipophilicity, facilitating its integration into biological membranes. This property is essential for maintaining cellular homeostasis and regulating cellular functions, including hematopoiesis (Cañete *et al.,* 2017; Ussishkin *et al.,* 2023). Retinol is instrumental in the production and differentiation of blood cells, directly influencing erythropoiesis and shaping the overall hematological profile (Restrepo-Gallego *et al.,* 2020; Amimo *et al.,* 2022), including red blood cell production and immune function. Retinol predominantly exists in the all-trans configuration, which is vital for its physiological effectiveness. Changes in stereochemistry can markedly affect its binding affinity to nuclear receptors, subsequently modifying its regulatory functions. The conversion of retinol into retinaldehyde and retinoic acid highlights its dynamic role in retinoid

signaling pathways. Notably, retinoic acid is a potent regulator of gene expression involved in hematopoiesis, thereby impacting key hematological parameters and promoting immune responses.

In the bloodstream, retinol is transported bound to retinol-binding protein (RBP), which plays a crucial role in delivering retinol to target tissues (Steinhoff *et al.,* 2022). This binding is essential as it prevents the potential adverse effects of unbound retinol, which can disrupt cellular membranes and compromise overall cellular health. Within cells, retinol associates with various intracellular RBPs, ensuring its availability for metabolic activities critical for maintaining tissue function (Est and Murphy, 2023). Retinol can also undergo esterification (O'Byrne *et al.,* 2013) to form retinyl esters, the primary storage form of vitamin A in various tissues. This process enhances the stability and lipophilicity of these esters, making retinyl palmitate and other derivatives the predominant forms found in dietary supplements and fortified foods (Scientific Committee on Consumer Safety, 2022). These esters are mainly stored in the liver and released as needed, ensuring a reliable supply of retinol for critical physiological functions (Czuba *et al.,* 2020).

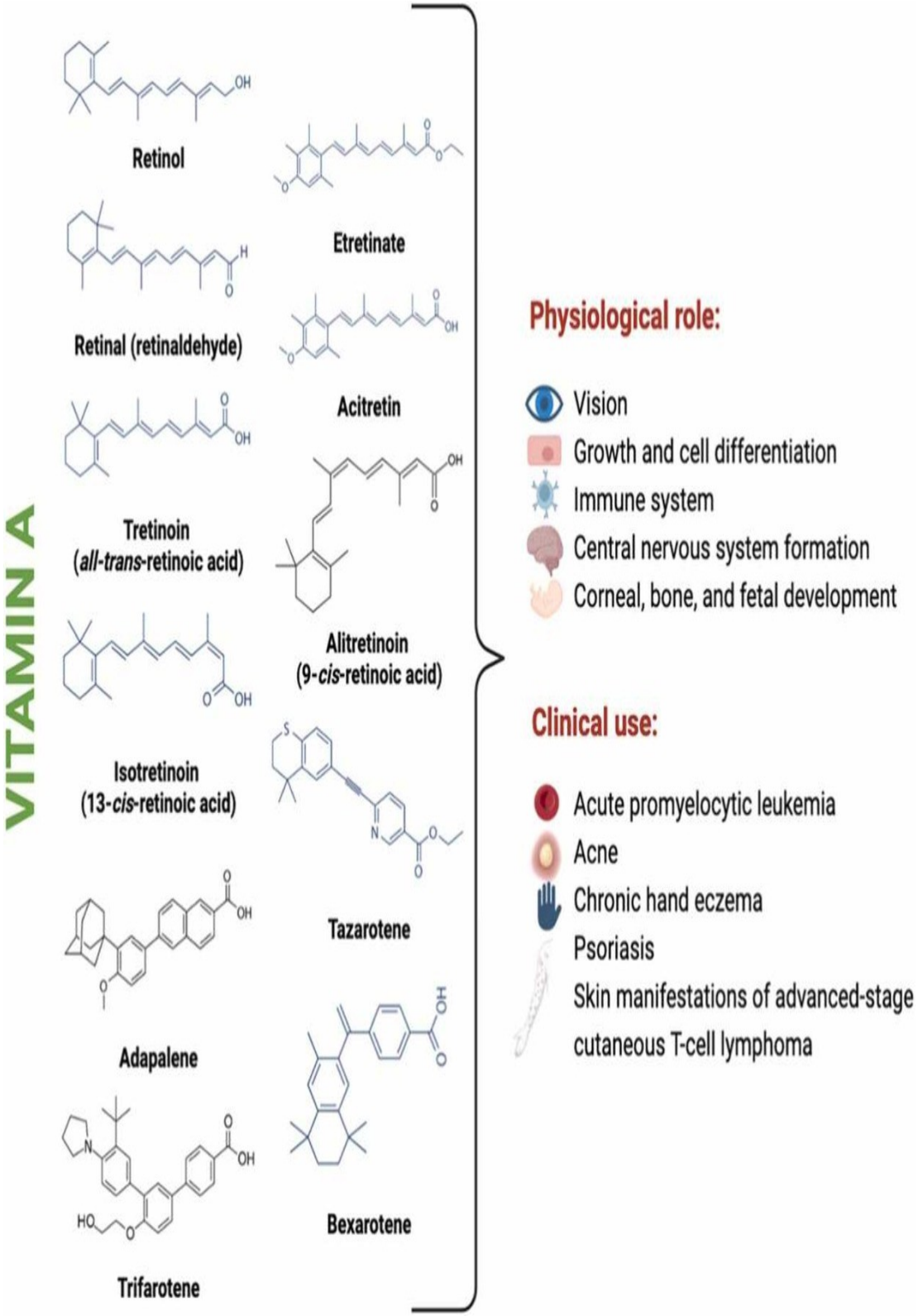

Figure 2.4: Retinol significance and structure (Wang *et al.,* 2020).

From a histological perspective, retinol is crucial for cellular differentiation and proliferation, particularly during embryonic development and tissue maintenance (Wang *et al.,* 2020). The action of retinoic acid in regulating gene expression related to tissue morphogenesis and differentiation significantly influences the structure and function of various organs (Jia and Bi, 2023), highlighting its importance in maintaining tissue integrity (Cunningham *et al.,* 2015; Lavudi *et al.,* 2023). The structural characteristics of retinol not only define its essential nutrient role but also underscore its significance in hematological parameters and histological changes. Highlighting these structural and functional relationships provides valuable insights into retinol's mechanisms of action and potential therapeutic applications, especially during pregnancy. While adequate retinol intake is vital for healthy hematopoiesis and tissue development., its multifaceted nature emphasizes the necessity for continued research into retinol's diverse roles in health and disease, particularly regarding its impact on serum proteins and overall physiological well-being (Kawczak*et al.,* 2024)

2.2 Pharmacokinetics of Retinol (Vitamin A)

The pharmacokinetics of retinol involves its absorption, transport, and distribution throughout the body, which are essential for delivering it to tissues where it can be converted into active metabolites to carry out vital biological roles (Sugandhi *et al.,* 2023). The upcoming sections will explore each stage of retinol's movement and processing in detail.

2.2.1 Absorption of retinol (vitamin a):

The absorption of vitamin A, primarily derived from dietary retinol and carotenoids, is a complex and regulated process which is influenced by various factors, including

the source of vitamin A, the presence of dietary fats, and the health of the gastrointestinal system (Harrison, 2011; Carazo *et al.,* 2021). Vitamin A is mainly obtained from dietary sources, with animal-based foods providing preformed vitamin A (retinol) and plant-based foods contributing provitamin A carotenoids, which must be converted into retinol within the body (National Research Council, 1989).

Retinol, often esterified to retinyl esters in food sources, undergoes hydrolysis in the stomach by gastric lipases, releasing free retinol (D'Ambrosio *et al.,* 2011). This free retinol is absorbed primarily in the small intestine, particularly the duodenum and jejunum. Absorption involves both passive diffusion and active transport mechanisms (Alagga *et al.,* 2024). Retinol's incorporation into mixed micelles in the intestinal lumen, facilitated by bile salts and dietary fats, enhances its solubility and absorption. The active transport of retinol into enterocytes is primarily mediated by specific membrane transporters, such as the retinol transporter STRA6 and retinol-binding proteins (Dhokia and Macip, 2021). STRA6 facilitates the uptake of retinol from the bloodstream into enterocytes, where RBP plays a critical role in binding and stabilizing retinol within the cell. Once inside, retinol is either stored or converted into its active form, retinoic acid, for further cellular processes (Zhong *et al.,* 2020).

Within enterocytes, retinol binds to cellular retinol-binding protein (CRBP), which is crucial for its intracellular transport and stabilization for further metabolism (Bohn *et al.,* 2019). CRBP1, as the major intracellular retinol-binding protein, facilitates efficient retinoid use by providing a sink that promotes retinol uptake from sRBP through the plasma membrane or via STRA6, and directs retinol or retinal to enzymes involved in the synthesis of retinyl esters or retinoic acid (O'Connor *et al.,* 2022). It also helps protect retinol/retinal from excessive breakdown or unwanted metabolism.

Additionally, intracellular retinoic acid-binding proteins (CRABP1 and 2, as well as FABP5) contribute to diverse functions by guiding retinoic acid toward catabolism, directing it to nuclear receptors, and regulating non-canonical actions (Park *et al.,* 2019). These binding proteins are critical to maintaining retinoid homeostasis. Gene ablation of CRBP1, CRBP2, and CRBP3 does not result in gross developmental defects, but metabolic and functional abnormalities highlight their importance for health, particularly CRBP2's role in survival during vitamin A deficiency (Nagpal *et al.,* 2019). Further research is necessary to uncover the precise molecular interactions between these binding proteins and their targets, which are essential for retinoid metabolism and function (Napoli, 2016).

The efficiency of retinol absorption is closely tied to the presence of dietary fat, which promotes the formation of micelles that facilitate the absorption of fat-soluble vitamins like retinol (Sugandhi *et al.,* 2023). Additionally, essential micronutrients such as zinc play a key role in optimizing retinol absorption by contributing to the synthesis of retinol-binding proteins and enzymes critical for retinol metabolism. Carotenoid absorption, although less efficient, also occurs primarily through passive diffusion, with specific transport proteins such as CD36 and scavenger receptor class B1 (SCARB1) assisting their uptake (Von Lintig *et al.,* 2019). Once inside enterocytes, carotenoids like β-carotene can be converted to retinol, with conversion efficiency influenced by dietary fat content and existing retinol levels in the body. Disruptions in gastrointestinal function, such as conditions affecting lipid digestion or deficiencies in zinc, can significantly impair retinol absorption, leading to suboptimal vitamin A status (Lauridsen *et al.,* 2021; Jackson *et al.,* 2023). Thus, the bioavailability and efficient absorption of retinol depend on a synergistic interaction

between dietary composition, micronutrient availability, and gastrointestinal health (Carazo *et al.,* 2021).

2.2.2 Transport and distribution of retinol (Vitamin A):

Following absorption into enterocytes, retinol is esterified to retinyl esters by the enzyme acyl-CoA:retinol acyltransferase (ARAT) and subsequently incorporated into chylomicrons for transport via the lymphatic system to the liver and other peripheral tissues (Gudas, 2022). Chylomicrons, composed of triglycerides, phospholipids, proteins, and cholesterol, are critical for the transport of fat-soluble vitamins, including retinol. Upon reaching the bloodstream, chylomicron remnants are taken up by liver cells, where they are processed (Sholola *et al.,* 2022). In the liver, retinyl esters are hydrolyzed back to retinol, which then binds to retinol-binding protein (RBP), forming a complex that serves as the primary transporter of retinol throughout the bloodstream (Abbott-Johnson *et al.,* 2014). The retinol-RBP complex ensures efficient distribution of retinol to various tissues, including the kidneys, lungs, and bone marrow, where it plays essential roles in physiological processes (Dhokia *et al.,* 2021). Retinol is also stored in the liver as retinyl esters, and its release into circulation is tightly regulated according to the body's needs. Transthyretin (TTR), a stabilizing protein that binds to the retinol-RBP complex, prevents renal clearance of retinol, ensuring its continued availability for tissue delivery (Cleveland Clinic, 2024). The efficiency of retinol transport is influenced by factors such as RBP levels and the presence of other binding proteins (Bohn *et al.,* 2019).

During periods of vitamin A deficiency, retinol is mobilized from liver stores and distributed to critical tissues. When vitamin A stores are sufficient, retinol is preferentially retained in the liver for future use. In addition to the liver, other tissues

such as adipose tissue, kidneys, and bone marrow also receive retinol as needed for various cellular and metabolic functions (Carazo *et al.,* 2021). Following absorption, retinol is converted into retinyl esters in enterocytes before being secreted into the lymphatic system encapsulated in chylomicrons. These chylomicrons undergo enzymatic hydrolysis, forming chylomicron remnants that are taken up by the liver. Within the liver, retinol is reconverted into retinyl esters by lecithin retinol acyltransferase (LRAT), primarily in hepatic stellate cells, and stored for future release. When needed, retinyl esters are hydrolyzed back to retinol, which then binds to RBP for systemic distribution (Steinhoff *et al.,*2022; Sugandhi *et al.,* 2023).

The retinol-RBP complex circulates in plasma and delivers retinol to various tissues such as the kidneys, bone marrow, and adipose tissue (Carazo *et al.,* 2021). Specific transporters on cell surfaces ensure that retinol enters cells, where it is utilized for essential functions like gene regulation, cellular differentiation, and immune modulation (Kim *et al.,* 2022; Shastak *et al.*, 2024). Under normal conditions, retinol delivery is maintained at stable levels. However, in vitamin A deficiency, reduced RBP levels hinder retinol transport, leading to insufficient vitamin A availability in tissues. In pregnancy, vitamin A is transferred from the mother to the fetus via the placenta (Quadro *et al.,* 2024). After birth, retinol is also transferred to the infant through breast milk (Mikles, 2024; McLaughlin, 2024). A reduction in retinol levels during the third trimester of pregnancy can result in decreased hepatic vitamin A reserves in newborns, helping to minimize the teratogenic risks associated with excessive vitamin A (Maia *et al.,* 2019; Gannon *et al.,* 2020). Postnatally, breast milk provides additional vitamin A to infants, further emphasizing the importance of maternal vitamin A status during pregnancy and lactation (World Health Organization [WHO], 2023).

2.2.3 Regulation of retinol transport:

Retinol transport and distribution are influenced by various factors, including nutritional status, hormonal regulation, and genetic factors (Reay *et al.,* 2024). During periods of vitamin A deficiency, the body upregulates the synthesis of RBP to ensure adequate retinol delivery to tissues. Conversely, excess vitamin A intake results in the downregulation of RBP synthesis, preventing the toxic accumulation of retinol (O'Connor *et al.,* 2022) Moreover, the hepatic synthesis of retinol-binding proteins is regulated by retinoic acid, the active metabolite of retinol, which interacts with nuclear receptors to modulate gene expression involved in vitamin A metabolism and transport (Carazo *et al.,* 2021; Steinhoff *et al*., 2022).

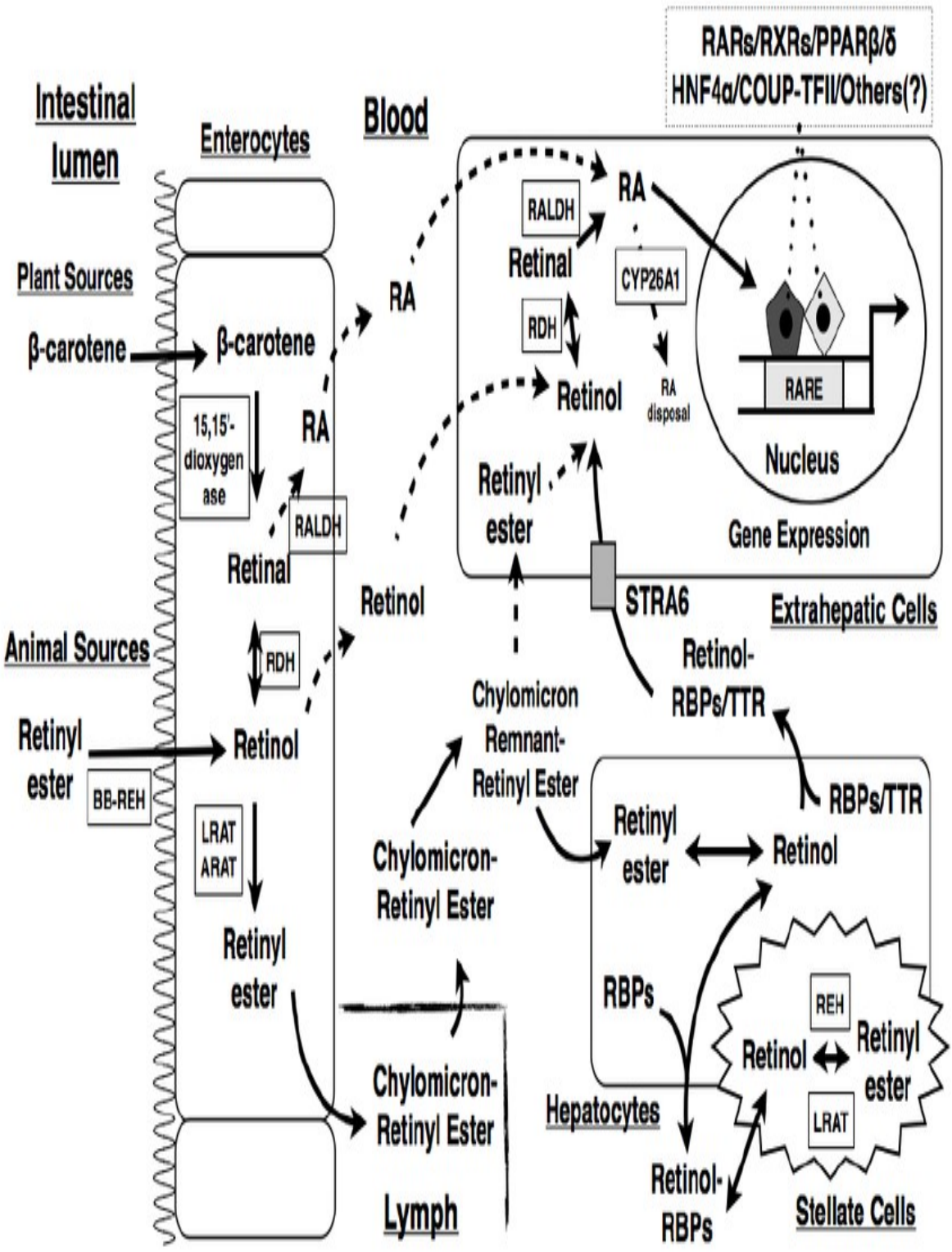

Figure 2.5: Pharmacokinetics of Vitamin A (Retinol) ADME

2.3 The Metabolism of Retinol (Vitamin A)

Vitamin A metabolism involves several stages, including the storage of retinol in the liver, its conversion to active metabolites, and the elimination of excess through excretion. These processes are crucial for maintaining the proper balance of retinoids in the body to support essential physiological functions.

2.3.1 Storage of vitamin A:

The metabolism of vitamin A involves a complex interplay among its various forms, including retinal, retinol, and retinoic acid, facilitated by carrier proteins and enzymes. In humans, vitamin A is primarily stored in the liver as retinyl esters in hepatic stellate cells (Chen *et al.,* 2023). These cells, specialized in the storage of fat-soluble vitamins, can release the stored retinol as needed, ensuring the body's vitamin A requirements are met during periods of low dietary intake (Manetti *et al.,* 2023; Lerner, 2024). The liver serves as the central reservoir for vitamin A, maintaining substantial reserves sufficient for several months under normal physiological conditions (Saeed *et al.,* 2017; Hakeem *et al.,* 2024). Vitamin A must be obtained through the diet, primarily from preformed vitamin A found in animal products and provitamin A carotenoids from plant sources. Preformed vitamin A is found in foods such as dairy products, liver, fish oils, and human milk. Provitamin A carotenoids, such as β-carotene and α-carotene, are organic pigments produced by plants, algae, and certain bacteria, contributing to the vibrant colors of many fruits and vegetables (Arruda *et al.,* 2022). These carotenoids are classified into two types: carotenes, which lack oxygen, and xanthophylls, which contain oxygen (González-Peña *et al.,* 2023). The bioavailability and absorption of vitamin A from plant sources tend to be lower than from animal sources, highlighting the importance of animal-based foods in maintaining adequate

vitamin A levels (Chungchunlam *et al.,* 2023). Specific carotenoids, notably β-carotene, exhibit provitamin A activity, enabling conversion to vitamin A in the body. Since mammals cannot synthesize carotenoids, they must rely on animal-based sources or plant-based precursors (Harrison, 2019). In the digestive system, β-carotene undergoes oxidative cleavage to form all-trans-retinal, which can then be further converted into retinol or retinyl esters (Bohn *et al.,* 2019). This process is essential for the formation of retinyl esters, which serve as the key storage form of vitamin A. The reduction of all-trans-retinal results in the formation of all-trans-retinol, which is esterified to form all-trans-retinyl esters crucial transport and storage forms of vitamin A in mammals (Dewett *et al.,* 2021). Furthermore, the absorption and digestion of vitamin A are closely linked to lipid absorption (Harrison, 2011). A diet deficient in fat (below 5–10 g/day), or conditions such as pancreatic or liver diseases and recurrent gastroenteritis, can impair lipid digestion, leading to steatorrhea and reduced vitamin A absorption (Zuvarox *et al.,* 2023).

2.3.2 Conversion of retinol:

Once absorbed and transported to target tissues, retinol undergoes a series of enzymatic transformations to yield its active metabolites, which are critical for its biological functions. The initial step in this metabolic pathway involves the conversion of retinol to retinal, catalyzed by alcohol dehydrogenases (ADHs) (Thompson *et al.,* 2019). This transformation primarily occurs in the liver and peripheral tissues (Napoli and Yoo, 2020). Retinal, the aldehyde form of vitamin A, serves as an intermediary metabolite in the conversion of retinol to retinoic acid. Subsequent to its formation, retinal undergoes further oxidation by retinaldehyde dehydrogenases (RALDHs), enzymes found in tissues such as the liver, lungs, and

kidneys, to produce all-trans retinoic acid (ATRA), the biologically active form of retinol. This conversion is a key step, as ATRA is central to various physiological processes (Kedishvili, 2016; O'Connor *et al.,* 2022). ATRA plays a pivotal role in regulating gene expression, immune modulation, cellular differentiation, and apoptosis. It acts as a ligand for retinoic acid receptors (RARs) and peroxisome proliferator-activated receptors (PPARs), which in turn initiate the expression of genes involved in immune system regulation (Czarnewski *et al.,* 2017; Wei *et al.,* 2023), vision, and embryonic development. Given the critical functions of ATRA, its levels are tightly regulated. Excessive accumulation of ATRA can lead to cellular toxicity, emphasizing the need for precise control over its synthesis and activity (Chen *et al.,* 2024). Enzymes involved in the conversion of retinol are subject to feedback inhibition, and their activity is influenced by dietary factors, such as carotenoids, as well as hormonal regulators, including thyroid hormones, which modulate the enzymes responsible for retinol conversion (Gudas, 2022). This regulatory system ensures that retinoic acid levels remain within a range that supports normal physiological processes without triggering toxic effects (Olson *et al.*, 2023).

2.3.3 Excretion and elimination of retinol and its metabolites:

Retinol and its metabolites are primarily excreted through the urine, bile, and feces (Molavi *et al.,* 2022). Retinoic acid, the active form of vitamin A, is conjugated with glucuronic acid by UDP-glucuronosyltransferases in the liver (Fujiwara *et al.,* 2018). This conjugation increases its water solubility, facilitating excretion primarily via the urine (Menezes *et al.,* 2024). Smaller amounts of retinoic acid are excreted via the bile. Once in the bile, retinoic acid and its conjugates can either be excreted in the feces or reabsorbed into the intestines, where they may be recirculated via the

enterohepatic circulation (Carazo *et al.,* 2021). While the kidney plays a role in excreting retinoic acid metabolites, the liver is the key organ involved in metabolizing and clearing retinol and its derivatives (Gudas, 2022). Retinol and retinoic acid are fat-soluble compounds, meaning they can be stored in the liver for long periods, reducing the need for continuous intake (Reddy *et al.,* 2022). However, this also means that excessive intake can lead to toxic buildup, particularly if the body's regulatory systems are overwhelmed. Hypervitaminosis A can occur when excessive amounts of preformed vitamin A are consumed over prolonged periods, leading to symptoms such as nausea, dizziness, liver damage, and teratogenicity during pregnancy (Olson *et al.,* 2023). The half-life of retinoic acid in circulation is relatively short, making adequate intake essential to maintain appropriate biological activity (O'Byrne *et al.,* 2013). This highlights the importance of monitoring vitamin A intake, especially for pregnant women and individuals using retinoid-based therapeutics.

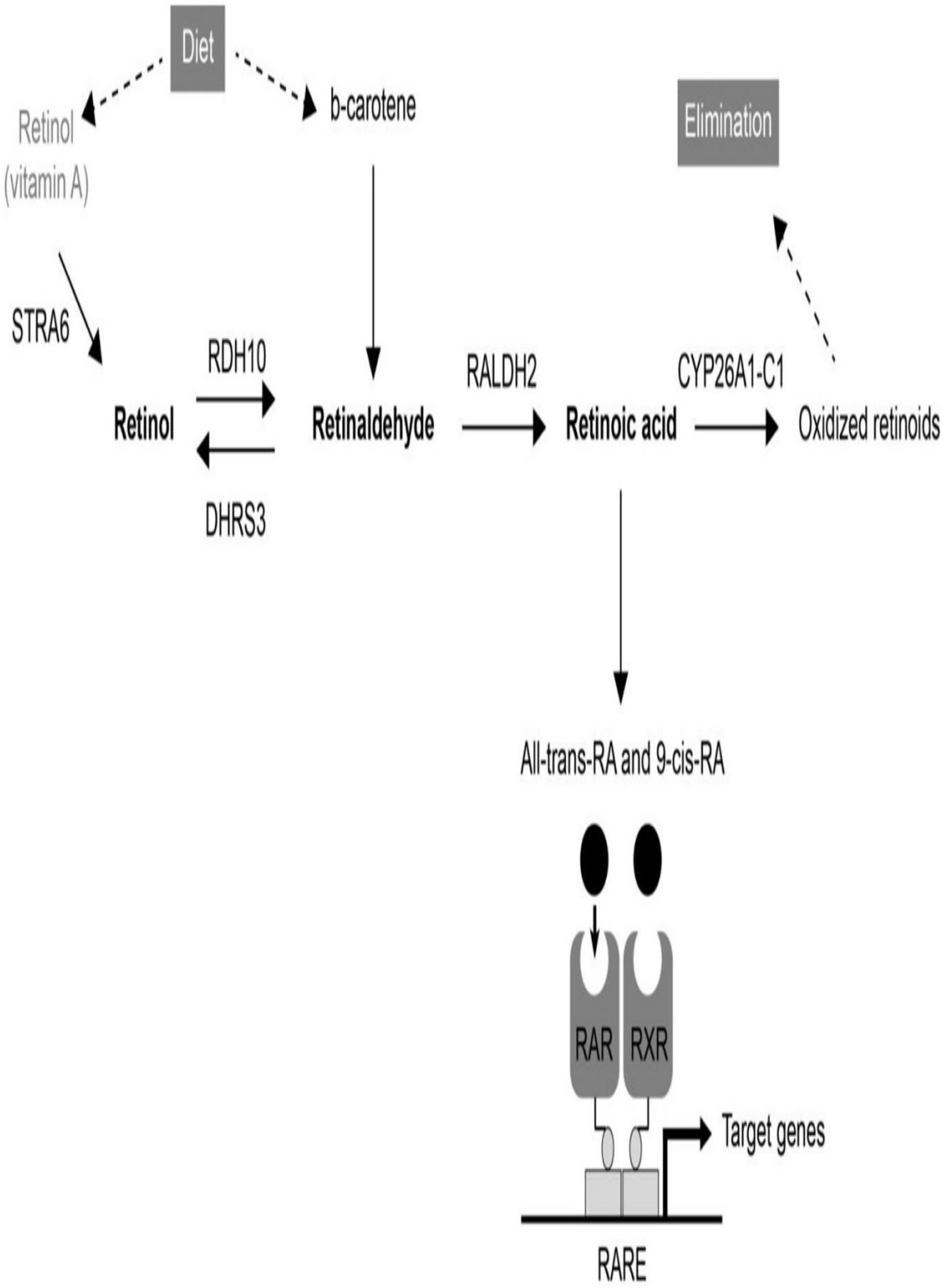

Figure 2.6: Retinol Metabolism and Retinoic Acid Pathway

2.4 Mechanisms of Action

Retinol exerts its effects through various mechanisms, including the regulation of gene expression, support of immune function, provision of antioxidant activity, promotion of cellular differentiation and hematopoiesis. Each of these mechanisms plays a vital role in maintaining physiological health and facilitating development.

2.4.1 Retinol action in gene regulation:

Retinol acts as a precursor to retinoic acid, which is a biologically active metabolite crucial for the regulation of gene expression in the body (Ahmed, 2023). Retinoic acid regulates gene expression by activating nuclear retinoic acid receptors (RARs) and retinoid X receptors (RXRs), influencing the transcription of genes involved in cell differentiation, growth, and development (Sharma *et al.,* 2022; Lavudi *et al.,* 2023). The interaction between retinoic acid and these nuclear receptors is fundamental to gene regulation. When retinoic acid binds to RARs, it triggers a conformational change that activates the receptor. The activated RAR can pair with RXRs to form a heterodimer, which is more effective at binding to specific DNA sequences known as retinoic acid response elements (RAREs). Once this RAR-RXR complex is formed and bound to RAREs, it can recruit additional proteins called coactivators which include the p160 subfamily of steroid receptor coactivators (SRC), namely, SRC-1 (also referred to as NCoA-1), SRC-2 (TIF-2, GRIP-1), and SRC-3 (pCIP, ACTR, AlB1, TRAM1, RAC3), (Lefebvre *et al.,* 2005; Al Tanoury *et al.,* 2013) that plays crucial role in promoting the transcription of specific target genes. Coactivators achieve this by adding acetyl groups to histones, the proteins around which DNA is wrapped. This modification, known as histone acetylation, leads to a more relaxed chromatin structure, making the DNA more accessible to the transcription machinery

of the cell. With the DNA in a more open conformation, the transcription machinery, including RNA polymerase II, can easily access the genes that need to be transcribed, synthesizing messenger RNA (mRNA) from the DNA template. This entire process is essential for various biological functions, including development and cellular differentiation, particularly during critical periods like pregnancy, where precise gene regulation is necessary for normal physiological function (Donovan *et al.,* 2022).

In contrast, in the absence of retinoic acid, RARs can recruit corepressor proteins complex such as NCOR/Sin3A/HDAC in the left side of the nucleus that inhibit gene transcription by promoting a closed chromatin conformation, thus restricting access to the DNA. This dual regulatory mechanism enables retinoids to toggle between activating and repressing gene expression based on the presence of retinoic acid (Cunningham *et al.,* 2015). The balance between coactivators, which facilitate transcription, and corepressors, which inhibit it, ensures that genes are turned on or off as needed, depending on the cellular environment and conditions. Retinoic acid signaling also involves chromatin remodeling complexes, such as SWI/SNF, which alter the positioning of nucleosomes, structures made up of DNA and histones (Mardinian *et al.,* 2021). By repositioning nucleosomes, these complexes enhance DNA accessibility, allowing transcription factors and RNA polymerase II to form the transcription preinitiation complex (PIC). This complex is essential for initiating the transcription of retinoid-responsive genes (Schier and Taatjes, 2020).

Phosphorylation is another crucial process that regulates the activity of retinoic acid receptors. The addition of phosphate groups to RARs can enhance or inhibit their ability to activate gene transcription, affecting their interactions with coactivators and corepressors (Lavudi *et al.,* 2023). Additionally, the controlled degradation of retinoic

acid receptors through the ubiquitin-proteasome system maintains proper receptor activity levels, ensuring tight regulation of gene expression (Kang *et al.,* 2022). Retinoic acid signaling is interconnected with various other signaling pathways in the body. For example, retinoid receptors can collaborate with other nuclear receptors, such as peroxisome proliferator-activated receptors (PPARs), creating a complex network that enhances gene regulation. This cross-talk is vital for integrating the functions of retinoids with metabolic and developmental pathways, allowing retinoic acid to influence numerous physiological processes (Kang *et al.,* 2022).

Overall, the physiological implications of retinoic acid extend to critical roles in various developmental processes. It is essential for organ formation, neuronal differentiation, and the establishment of body patterns during embryonic growth (Jia & Bi, 2023). By regulating the expression of important genes, including HOX genes vital for limb, organ, and central nervous system development, retinoic acid significantly impacts overall biological function (Hubert and Wellik, 2023). As a precursor to retinoic acid, retinol influences gene regulation through this complex mechanism involving nuclear receptors, chromatin remodeling, and coactivator-corepressor dynamics, as well as post-translational modifications like phosphorylation (Al Tanoury et al; 2013; Sharma *et al.,* 2022). This intricate network highlights the critical role of retinoids in developmental biology and their broader implications for health, particularly during crucial stages of development when precise regulation is essential for normal physiological outcomes (Dubey *et al.,* 2018; Margiana *et al.,* 2022).

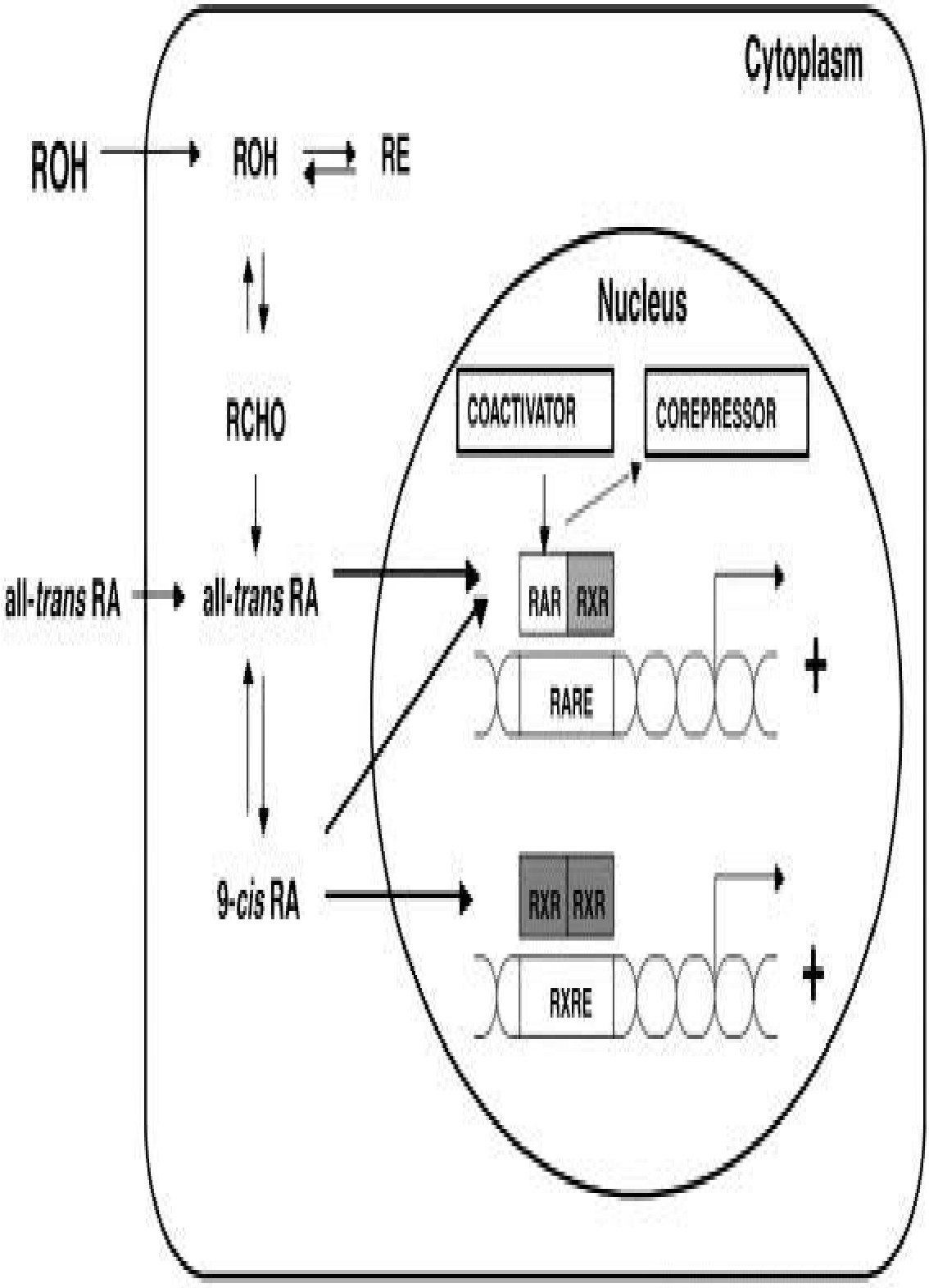

Figure 2.7: Vitamin A Regulation of Gene Expression (Sharma *et al.,* 2022)

2.5 Retinol action in hematopoiesis:

Retinol, through its active metabolite retinoic acid (RA), plays a pivotal role in regulating hematopoiesis, significantly influencing various stages of blood cell development from embryogenesis to adulthood (Hawkins *et al.,* 2023). The importance of retinol in this process begins during early embryonic development, where RA signaling is crucial for the emergence of definitive hematopoietic stem cells (HSCs) (Cañete *et al.,* 2017). This significance is particularly evident in the aorta-gonad-mesonephros (AGM) region, where the enzyme retinaldehyde dehydrogenase 2 (RALDH2) is expressed in the intermediate mesoderm (Heck *et al.,* 2020). RALDH2 activity is essential for hematopoietic lineage specification, highlighting how RA is intricately involved in the regulation of the endothelial cell cycle and the specification of hemogenic endothelium, which serves as a precursor to HSCs (Wu and Hirschi, 2020).

In the fetal liver, retinoic acid is vital for regulating gene expression linked to HSC identity and function. Research has demonstrated that RA signaling contributes to the induction of HOXA gene expression, essential for maintaining proper HSC function (Steens and Klein, 2022). Furthermore, during the early phases of erythropoiesis, retinoic acid signaling acts in concert with erythropoietin (EPO) to promote red blood cell production, underscoring its role in the transition from yolk sac to fetal liver hematopoiesis (Cañete *et al.,* 2017; Bhoopalan *et al.,* 2020). The placenta and umbilical cord also exhibit hematopoietic activity, where the expression of RA signaling components, including RXRα, suggests a potential role in placental hematopoiesis (Chia *et al.,* 2021). Although direct evidence of RA's role in this context remains to be fully elucidated, the presence of hemogenic precursors in the

placental labyrinth indicates that further investigation is warranted in this area (Weijts and Robin, 2024).

Zebrafish models have provided insights into the role of retinoic acid in hematopoiesis, demonstrating that RA signaling is necessary for the expression of HSC markers prior to the formation of the aorta (Hawkins *et al.,* 2023). Additionally, RA has been shown to regulate primitive waves of erythropoiesis and myelopoiesis, emphasizing its dual role in promoting and inhibiting different aspects of hematopoietic development (Cañete *et al.,* 2017). In adults, retinol and its derivatives are critical for maintaining hematopoietic system homeostasis. RARα and RARγ receptors are prominently expressed in various bone marrow cells, with RARγ playing a key role in regulating lymphocyte differentiation. Evidence suggests that retinoic acid signaling mediates the modulation of granulocyte and monocyte differentiation, illustrating its ongoing influence throughout the lifespan (Cañete *et al.,* 2017; Brown, 2023). Moreover, the influence of retinol on hematopoiesis extends beyond direct transcriptional regulation to include epigenetic mechanisms. Aberrant chromatin configurations induced by oncoproteins in hematopoietic malignancies illustrate the complexity of RA's role in both normal and malignant hematopoiesis, particularly in resistance to treatments such as acute myeloid leukemia (AML), (Hu *et al.,* 2021). The interplay between retinoic acid and chromatin-modifying enzymes, including DNA methyltransferases, underscores the intricate regulation involved in hematopoiesis. Furthermore, the interrelationship between vitamin A and vitamin D signaling pathways highlights their collective influence on hematopoiesis (Geoffroy *et al.,* 2021) While vitamin D is not strictly necessary for hematopoiesis, it modulates various processes and can enhance the effects of retinoic acid on differentiation (Arora *et al.,* 2024). This complex network of interactions emphasizes the importance

of retinol in both embryonic and adult hematopoiesis and continued research into these pathways may provide novel approaches for addressing hematopoietic malignancies and related conditions (Lavudi et al., 2023).

2.6 The influence of retinol on hematological parameters during pregnancy

Several studies has been carried out on animal models and it has been seen that, Pregnancy triggers significant physiological adaptations in the maternal body, particularly in hemostasis, immune function, and overall maternal health. These changes are driven by the metabolic demands of the growing fetus, the mother's preparations for childbirth, and the necessary adjustments to support fetal development and ensure successful delivery. Key physiological processes involved in these adaptations include alterations in blood volume, immune responses, haematological parameters, and the body's ability to manage increased circulatory load. In both humans and animal models like Wistar rats, understanding these changes is crucial for monitoring maternal health, identifying complications, and optimizing pregnancy outcomes (Vinnars *et al.,* 2023). One of the most notable changes during pregnancy is the expansion of plasma volume, which increases by 40-50% in both humans and Wistar rats (Aguree *et al.,* 2019). This expansion, regulated by hormonal factors such as estrogen and progesterone, supports fetal growth by enhancing uteroplacental blood flow, ensuring efficient nutrient and oxygen delivery to the developing fetus, and compensating for potential blood loss during delivery. Plasma volume expansion is essential to meet the growing metabolic needs of the mother and fetus but can have unintended consequences. It results in dilutional anemia, a condition in which the increased blood plasma dilutes the concentration of red blood cells (RBCs), lowering hemoglobin and hematocrit levels. This dilution is most

pronounced during the second trimester, when plasma volume expansion peaks. The reduction in RBC count and hemoglobin is typically mild, but it requires careful monitoring to prevent complications like iron deficiency anemia. While plasma volume expansion contributes to RBC dilution, it also stimulates an increase in RBC mass due to elevated erythropoietin levels, a hormone produced by the kidneys in response to hypoxia. However, the increase in RBC mass is smaller than the increase in plasma volume, resulting in a net decrease in RBC concentration (Cleveland Clinic, 2024). Changes in mean corpuscular volume (MCV) and other RBC indices, such as mean corpuscular hemoglobin (MCH), can also reflect the production of larger, younger RBCs, known as macrocytosis. A decrease in MCV below 84 fL, accompanied by low hemoglobin levels, is often a sign of iron deficiency, which is common in pregnancy due to increased maternal iron demands. Postpartum, plasma volume typically decreases, and blood parameters generally return to pre-pregnancy levels within 6-8 weeks. However, it is important to note that iron deficiency anemia remains a significant concern in pregnancy and should be addressed through appropriate nutritional supplementation (Abu-Ouf *et al.,* 2015; Georgieff, 2020). Pregnancy is also characterized by leukocytosis, or an increase in white blood cell (WBC) count, driven by the immune demands of pregnancy. The immune system undergoes adaptations to tolerate the growing fetus while protecting the mother from infections. In humans, WBC counts rise steadily throughout pregnancy, particularly due to an increase in neutrophils, which play a key role in the immune response to infection (Paradise, 2024; Zhu *et al.,* 2024). In rats, similar changes occur, including the appearance of immature neutrophils and toxic granulation indicators of heightened immune activity. Additionally, lymphocyte counts typically decrease early in pregnancy but rise again in the third trimester as part of the immune adaptations

needed to protect the fetus (Abu-Raya *et al.,* 2020). Monocyte counts increase early in pregnancy to maintain immune tolerance and prevent fetal rejection. Both species demonstrate a rising monocyte-to-lymphocyte ratio, which reflects shifts in immune regulation. Eosinophils and basophils remain relatively stable during pregnancy (Yıldırım *et al.,* 2024). These changes in WBC profiles are critical for maintaining immune function and pregnancy progression. WBC counts peak immediately postpartum, reaching as high as 25,000 cells per cubic millimeter, and return to baseline within 4 weeks (Zhu *et al.,* 2024).

Gestational thrombocytopenia, or a decrease in platelet count, is another common hematological change in pregnancy, particularly during the third trimester. This reduction in platelet count is attributed to hemodilution (due to plasma volume expansion), increased platelet activation, and faster platelet turnover (Madormo, 2023). Although platelet counts decrease, the reduction is usually mild and does not pose major complications (UT Southwestern Medical Center, 2021; Kumar, 2024). Platelet volume distribution width (PDW) increases, reflecting the elevated turnover of platelets. Consequently, mean platelet volume (MPV) is less reliable as an indicator of platelet size during pregnancy (Gürsoy*et et al.,* 2022). Postpartum, platelet counts generally rise in response to platelet consumption during labor, which helps ensure adequate clotting and reduce the risk of hemorrhage. Vitamin A (retinol) plays an essential role in the synthesis of clotting factors II, VII, IX, and X in the liver, which are crucial for proper blood coagulation (van Dijk *et al.,* 2021). However, excessive intake of vitamin A can disrupt the production and metabolism of these clotting proteins, potentially impairing the body's ability to regulate bleeding and clotting. When consumed in high doses, vitamin A can amplify the anticoagulant effects of medications like warfarin (Coumadin), which works by inhibiting vitamin K-

dependent clotting factors, thereby reducing the blood's ability to clot. While vitamin A does not directly interfere with vitamin K, it can affect the liver's synthesis of clotting factors, potentially enhancing warfarin's anticoagulant effect. This may result in an increased risk of bleeding, with symptoms such as spontaneous bruising, frequent nosebleeds, or, in severe cases, internal bleeding (Tan *et al.,* 2020).

2.7 **The influence of retinol on biochemical parameters during pregnancy**

Retinol (vitamin A) plays a crucial role in maintaining biochemical homeostasis during pregnancy. As a fat-soluble micronutrient, retinol is involved in enzymatic reactions, gene expression regulation, and cellular differentiation all of which directly influence key biochemical parameters, especially in liver and kidney function, protein synthesis, lipid metabolism, and oxidative stress (Maia *et al.,* 2019). During pregnancy, the maternal liver adapts to increased metabolic demands by enhancing nutrient processing, detoxification, and protein synthesis. Because retinol is primarily stored in the liver, both deficiency and excess can significantly impair hepatic function. Elevated serum enzymes such as ALT, AST, and ALP often signal hepatocellular injury or cholestasis, which are documented effects of hypervitaminosis A. Conversely, retinol deficiency is associated with reduced protein synthesis, weakened antioxidant defenses (e.g., diminished glutathione activity), and lower production of retinol-binding proteins. The kidneys also play a critical role in retinoid metabolism and excretion. Elevated serum urea and creatinine levels have been reported following high-dose retinol supplementation, indicating nephrotoxicity marked by tubular degeneration or glomerular damage (Li *et al.,* 2021). Retinol additionally regulates lipid metabolism via PPARs and RXR signaling pathways, which are essential for lipid synthesis and degradation. In pregnant individuals,

imbalances in retinol can thus lead to altered cholesterol, triglyceride, and lipoprotein profiles, affecting maternal and fetal energy supply. From a chemical pathology perspective, monitoring biochemical markers such as liver enzymes, renal function tests, total protein, albumin, and lipid profiles provides early indicators of organ toxicity, metabolic disruption, and nutritional imbalance during pregnancy. These assessments offer a comprehensive view of the systemic impact of retinol and form a solid basis for defining safe and effective vitamin A consumption in maternal nutrition.

2.8 **Histological changes during pregnancy and its implications**

Pregnancy triggers significant structural changes in various organs to meet the demands of the growing fetus. These alterations are particularly evident in the liver, kidneys, uterus, and placenta. Animal models like Wistar rats are frequently used to study these changes, providing insight into similar processes in humans. In Wistar rats, pregnancy induces adaptations in several key organs. The liver often shows signs of hypertrophy, where liver cells (hepatocytes) enlarge to handle the increased metabolic needs related to nutrient processing and waste elimination (Garczyńska *et al.,* 2020). The kidneys also display hyperplasia, with an increase in cellular proliferation and adjustments in the structure of renal tubules and glomeruli to accommodate the rise in blood filtration. These changes help manage the higher blood volume and waste load during pregnancy. The uterus undergoes extensive remodeling, with smooth muscle cells becoming larger and more numerous (hyperplasia and hypertrophy) to support the growing fetus. There is also an increase in vascularization, improving blood flow to the uterine lining (Osol and Mandala, 2009). The placenta, crucial for fetal development, undergoes notable changes, including the expansion of trophoblast cells

and the formation of a dense network of blood vessels to optimize nutrient transfer between the mother and fetus (Tollefsbol, 2020). On a cellular level, pregnancy involves significant adjustments, particularly in the vascular and immune systems. In the liver, pregnancy can lead to increased lipid storage within hepatocytes (Sharma and John, 2024), while kidney tissues may exhibit enlarged glomeruli and tubules as they handle the increased filtration demands. These organs work together to maintain homeostasis and support the developing fetus. In the uterus, smooth muscle thickening is a common response to accommodate the fetus. The placenta, meanwhile, is continually remodeling, with trophoblast cells undergoing differentiation and proliferation, ensuring adequate blood and nutrient exchange. These structural changes are crucial for fetal growth and development. Retinol, which is converted into its active form, retinoic acid, plays a key role in regulating tissue differentiation and organ development during pregnancy. In Wistar rats, retinol has been found to influence the structure and function of several organ s, including the placenta, liver, and kidneys (Cañete *et al.,* 2017; Maia *et al.,* 2019). Retinoic acid affects gene expression related to growth and immune regulation, which is vital for ensuring healthy fetal development. In the placenta, retinol is necessary for the proper differentiation of trophoblast cells and the formation of a functional vascular network. However, excessive retinol intake can interfere with these processes. Research in pregnant rats has shown that high levels of retinol can lead to impaired trophoblast cell invasion and poor vascular development in the placenta. These changes may result in fetal growth restriction due to insufficient nutrient exchange, with the placenta exhibiting a smaller surface area in such cases (Roberts *et al.,* 2021). In the liver and kidneys, excessive retinol can cause tissue damage, including liver cell degeneration, fibrosis, and oxidative stress. These changes manifest as increased

inflammation, cell death, and tissue disruption in histological studies (Banerjee *et al.,* 2023). The kidneys may show glomerular sclerosis and tubular atrophy when exposed to excessive retinol, highlighting the importance of maintaining appropriate vitamin A levels during pregnancy (DiKun and Gudas, 2023). Aldh1a1, an enzyme responsible for converting retinol (vitamin A) into retinoic acid, plays a critical role in regulating several physiological processes, including reproductive functions (Thompson *et al.,* 2019). In the ovaries, Aldh1a1 is essential for the differentiation and proliferation of granulosa cells, which are vital for follicular development, ovulation, and luteal formation. Granulosa cells not only support the growth of follicles but also contribute to the secretion of steroid hormones such as estrogen and progesterone (Cai *et al.,* 2023). These hormones are crucial for sustaining pregnancy and promoting fetal development. Through its conversion of retinol to retinoic acid, Aldh1a1 influences various signaling pathways, including the PI3K-Akt and hedgehog pathways, which are essential for cellular proliferation, survival, and differentiation (Poturnajova *et al.,* 2021). When Aldh1a1 expression is increased, both of these pathways are enhanced, leading to greater granulosa cell proliferation and a subsequent rise in steroid hormone production.

2.9 Identification of Areas Where Results Are Inconsistent and Where More Research Is Needed

While substantial progress has been made in understanding retinol's role in pregnancy, particularly regarding its effects on some selected clinical laboratory parameters and histological outcomes in pregnant Wistar rats, several critical gaps persist. Adequate retinol intake is essential for fetal development and maternal health, enhancing clinical laboratory parameters such as red blood cell count, hemoglobin concentration,

hematocrit levels, Liver enzymes and renal markers. However, the optimal dosage required to achieve these benefits without causing toxicity remains uncertain. While higher doses have been linked to hepatic damage and teratogenic effects, some studies suggest potential benefits from increased intake, emphasizing the need for further research to establish a safe and effective dosage range during pregnancy. This study seeks to address these gaps by examining the dose-dependent effects of retinol on clinical laboratory parameters and histological changes in vital organs in pregnant Wistar rats. The findings will help define safe and effective supplementation guidelines to prevent both deficiency and toxicity, thereby improving maternal and fetal health outcomes. Moreover, this research will provide a basis for future studies on retinol's role in pregnancy, supporting the development of public health policies and global nutritional recommendations for pregnant women.

CHAPTER

RESEARCH METHODOLOGY

3.0 Study Area

This study was carried out in Ilorin, the capital of Kwara State, Nigeria, situated at approximately 8°30'N latitude and 4°30'E longitude. Ilorin is centrally located, about 300 km from Lagos and 500 km from Abuja, offering accessibility from different parts of the country. As a growing urban centre, Ilorin hosts several tertiary institutions and medical facilities, making it suitable for biomedical research. The availability of veterinary and laboratory services in the area provided essential support for the animal-based aspects of the study.

3.1 Study Design

This study employed a controlled laboratory-based experimental design utilizing a complete randomized block design to ensure a robust and objective comparison between treatment and control groups (Salinas Ruíz *et al.,* 2024). The female rats were systematically divided into two physiological blocks: pregnant and non-pregnant, based on their physiological status. Within each block, animals were randomly allocated to one of four distinct treatment groups. These groups included a control group receiving normal saline, and three experimental groups administered varying doses of retinol (minimal, therapeutic, and toxic/lethal). All treatments were administered orally via gavage once daily for 21 consecutive days to ensure precise dosing and consistent delivery. Following the dosing period, the animals were humanely sacrificed in accordance with ethical standards for the use and care of laboratory animals, and tissue samples were collected for subsequent hematological, biochemical, and histological analyses.

3.2 Study Population

The study population comprised a total of 40 Wistar rats, consisting of 32 female rats and 8 male rats. The female rats, serving as the primary experimental subjects, were carefully selected based on specific inclusion criteria: age between 12 to 14 weeks and body weights ranging from 200 to 300 grams. All animals were acquired from a certified laboratory animal breeding facility and confirmed to be healthy upon arrival. Wistar rats were chosen for this study due to their extensive use as a reliable and well-characterized model in biomedical research, particularly in pharmacology and toxicology studies, which facilitates comparison with a broad body of existing literature (Okafor *et al.,* 2021). The 8 healthy adult male rats were used exclusively for mating purposes to induce pregnancy in the female cohort and were not subjected to any treatment protocols or included in the subsequent outcome analysis.

3.3 Eligibility Criteria

To ensure the reliability and validity of the experimental outcomes, strict eligibility criteria were applied for the selection of animals used in the study.

Inclusion Criteria: Only female Wistar rats aged between 12 and 14 weeks were selected for the research. Eligible rats weighed between 200 and 300 grams and presented a healthy physical appearance, showing no signs of illness, deformities, or malnutrition. Animals had no prior exposure to vitamin A compounds or participation in any experimental procedures. For rats included in the pregnant subgroups, pregnancy was successfully confirmed through vaginal smear cytology prior to group assignment.

Exclusion Criteria: Male rats were excluded from the treatment arms of the study. Female rats younger than 12 weeks or older than 14 weeks, as well as those with body weights outside the 200–300 g range, were not considered eligible. Animals exhibiting clinical signs of disease, congenital abnormalities, previous experimental

involvement, or hypersensitivity to vitamin A derivatives were excluded. Rats with a history of reproductive failure or poor mating response were also not included in the pregnant group.

Additional Criteria: All selected rats were sourced from a certified laboratory animal breeding facility and acclimatized for a minimum of seven days prior to the start of the experiment. To reduce the influence of stress and environmental variability, animals were housed under standardized environmental conditions in individually ventilated cages with controlled lighting, temperature, and feeding protocols.

Withdrawal Criteria: Rats were withdrawn from the study if they showed signs of severe clinical distress, including rapid or excessive weight loss (more than 20%), failure to thrive, behavioral abnormalities, or adverse reactions to retinol treatment. Pregnant rats were also removed from the study if they failed to maintain pregnancy or showed signs of fetal resorption. All withdrawn were documented, and the affected rat were excluded from final data analysis.

3.4 Determination of Sample Size and Sampling Methodology

A total of thirty-two (32) female Wistar rats were utilized in the main experimental phase of this study. The sample size of four animals per group was determined based on established guidelines for rodent-based experimental research, which indicate that 4-5 animals per group are appropriate for achieving statistically viable results (Charan & Kantharia, 2013; Okafor *et al.,* 2021). This allocation resulted in a total of eight experimental groups, each containing four female rats. Sampling was conducted using a stratified randomization method. Within each physiological block (pregnant and non-pregnant), rats were randomly assigned to one of the four treatment subgroups (control, minimal, therapeutic, or toxic retinol doses). This approach ensured an equal distribution of animals across all treatment conditions within each physiological

category, thereby enhancing the internal validity of the experiment by accounting for pregnancy as a biological variable and minimizing potential selection bias.

3.5 Sample Size Calculation

The sample size for this study was determined using the Resource Equation Method, which is suitable for animal experiments when prior data on variability or effect size is unavailable (Arifin & Zahiruddin, 2017). This approach is ethically sound as it helps to avoid using an excessive number of animals.

The calculation is based on the animals used for treatment and analysis:

$E=N-G$ Where:

N = total number of female rats used for treatment and analysis (32)

G = number of treatment groups (8)

$E=32-8=24$.

An E value of 24 is statistically acceptable and reflects a well-powered design for detecting significant treatment effects. This calculation ensured both scientific rigor and ethical compliance by allowing for sufficient statistical analysis while avoiding the unnecessary use of animals.

3.6 Data collection procedures

Throughout the study, data were collected systematically to ensure accuracy and reliability. Body weight, food consumption, and water intake were monitored daily as indicators of general health and treatment-related effects, in line with established toxicological monitoring protocols (Niyomchan *et al.,* 2023). Pregnancy was confirmed by observing vaginal plugs the morning after mating and further supported by consistent maternal weight gain, which is a standard method for determining gestational day 0 in rodents (Ahloy-Dallaire *et al.,* 2019; Chesney *et al.,* 2020). Qualitative observations, such as lethargy, aggressiveness, or nesting behavior, were

also documented. Hematological indices (e.g., RBC count, WBC count, hemoglobin) were measured from whole blood using an automated hematology analyzer. Biochemical parameters relevant to liver and kidney function were measured from serum samples using standard assay kits. For histological analysis, tissue sections were stained with hematoxylin and eosin and examined microscopically for morphological alterations. All data were systematically recorded, classified, and stored to facilitate statistical analysis.

3.7 Sample Collection and Preparation

At the end of the 21-day experimental period, all rats were humanely anesthetized for sample collection in accordance with ethical standards (Parasuraman *et al.,* 2010; UCSF IACUC, 2022). Approximately 3–5 mL of blood was collected via jugular puncture into two types of tubes. For hematological analysis, a portion was immediately transferred into an EDTA-coated tube and gently inverted. The remaining blood was collected into a plain tube, allowed to clot at room temperature, and then centrifuged at 3000 rpm for 10 minutes to obtain serum for biochemical analysis. Immediately following euthanasia, the liver and kidneys were carefully excised using sterile dissection tools. Tissues were rinsed briefly in physiological saline to remove residual blood and placed in a labeled container for further processing.

3.8 Sample Storage

All biological samples were stored using established protocols to preserve their integrity. Whole blood in EDTA tubes for hematological assessment was refrigerated at 4°C and processed within 6 hours (Wu *et al.,* 2017; Unalli *et al.,* 2021). Serum was aliquoted into cryovials and stored at -20°C until biochemical analysis (Merck Veterinary Manual, 2025). The harvested liver and kidney tissues were fixed in 10%

neutral-buffered formalin for 24-48 hours to preserve cellular morphology for histopathological evaluation (Sakura Health Sciences *n.d.*; Magdeldin *et al.,* 2012).

3.9 Laboratory Analysis

3.9.1 Full blood count analysis using Sysmex Xp-300 analyzer

Procedure: Whole blood samples in EDTA tubes were thoroughly mixed by inversion before being analyzed using a Sysmex Xp-300 analyzer (Sysmex Corporation, Kobe, Japan). The full blood count results were generated approximately 60 seconds after the analysis began (Ezenwelu, 2007). The key parameters measured included White Blood Cells (WBC) and their differentials, including lymphocytes, neutrophils, monocytes, and eosinophils; and Platelet count (PLT).

3.9.2 Biochemical Analysis

For biochemical analyses, a semi-automated Mindray chemistry analyzer and a SpectraMax 250 UV-Visible microplate spectrophotometer (Molecular Devices) were utilized. Reagent and sample handling were performed using fixed-volume and adjustable micropipettes (20–1000 µL). A vortex mixer ensured uniform mixing of samples before analysis, while all required incubations were maintained at 37°C using both a digital incubator and a water bath. Assays were conducted in reaction tubes, microplates, and glass cuvettes, depending on the specific kit and analysis requirements. Serum samples were used to measure several key parameters, including Liver Function Tests (LFTs).

3.9.3 Histological Analysis

Rat necropsy

The rats were dissected using a student dissecting sets and the internal organ: lungs was removed carefully and prepared for histological studies. All animals were sacrificed by cervical dislocation at least three hours after the last exposure to smoke

extracts of cannabis and sterile cotton wool. They were laid down on the dissection board in a supine position and their anterior thoracoabdominal and pelvic wall with peritoneum was carefully dissected in the midline to expose the organ of interest. The lungs were transferred into 10% formol saline and were allowed to fix for at least 72 hours before further histological protocol and analysis.

1. Fixation

The fixative brings about crosslinking of proteins which produces denaturation or coagulation of proteins so that the semifluid state is converted into semisolid state; so that it maintains everything in vivo in relation to each other. Thus semisolid state facilitate easy manipulation of tissue.

2. Tissue Processing

The tissue processing is the heart of any tissue section which will be cut adequately only if the tissue is properly preserved and processed. It also refers to the treatment of the tissue necessary to impregnate it into a solid medium so that the tissue is rendered sufficiently firm yet elastic for the tissue sections of desirable thickness to be cut on microtome. STP 120 was used to process the tissue.

3. Embedding

It is the orientation of tissue in melted paraffin which when solidified provides a firm medium for keeping intact all parts of the tissue when sections are cut. The tissue is embedded in a solid medium by the help of first removing the tissue water which is then replaced by any solid medium such as paraffin wax so that the tissue is rendered firm enough to enable thin sections to be cut, at the same time, the tissue is soft (not so hard) to enable microtome knife to cut the sections. The tissue is embedded with the use of EC 350.

4. Sectioning

It involves the use of a precision machine called microtome to cut thin section that would allow light to pass through and aid microscopy. Rotary Microtome was used with precision of section set at 5um.

3.9.4 Histopathological Study

Fixed tissues cut to 4-6 mm were processed for Haematoxylin and Eosin staining. They were thoroughly rinsed in tap water, dehydrated and cleared of the dehydrating agent. They were infiltrated with wax and were made in blocks of wax for trimming and sectioning. Thin sections of 7 microns were stained with Haematoxylin and Eosin (H&E) and were studied histologically using Olympus binocular light research microscope (XSZ-107BN, No. 071771). Micrographs of the sections were taken with a Kodak Digital Camera (Kodak Easyshare C183) for subsequent histological analysis:

- The specimen was fixed in 10% buffered formol-saline
- The specimen was processed by method of dehydration through different grades of alcohol
- The specimen was de-alcoholised in clearing agent
- The specimen was infiltrated/ impregnated in paraffin wax
- The specimen was embedded in paraffin wax in cassette
- The specimen was sectioned at 4-5µm thickness on a rotary microtome
- The tissue sections was mounted onto a clean grease free glass slides and stretched using an albumin solution
- The slides were dried on an hot plate
- The specimen was stained by Haematoxylin and Eosin staining technique and viewed microscopically.

3.10 Haematoxylin and Eosin Staining Technique

3.10.1 Principle

The principle of H&E is based on Romanowsky principle, where Haematoxylin being a basic dye stains the acidic component of the tissue (Nucleus), and Eosin which is an acidic dye stains the basic component of the tissue (Cytoplasm).

3.10.2 Procedure

- The section was dewaxed in xylene
- The section was hydrated in descending grades of alcohols from absolute alcohol, 90% alcohols, 80% alcohol, and 70% alcohol
- The section was rinsed with water
- The section was stained/flooded with Haematoxylin for 5 minutes
- The section was rinsed with water
- The section was briefly differentiated in 1% acid alcohol.
- The section was rinsed with water
- The section was blued in water for 10 minutes
- The section was counter stained with Eosin for 2 minutes
- The section was rinsed with water
- The section was dehydrated in ascending grades of alcohol from 70%, 80%, 90% and absolute alcohol.
- The section was cleared in xylene and mounted in DPX.

Histological assessments was conducted using a light microscope for histological alteration description on the mounted slides. Finally, photomicrographs of the slides were taken using a camera.

3.11 Quality Assurance and Control

To ensure the validity, reliability, and accuracy of all laboratory data, a robust quality assurance program was maintained throughout the study. The Sysmex Xp-300

hematology analyzer and the Mindray chemistry analyzer were calibrated daily using manufacturer-supplied control materials, with all calibration values verified to be within specified limits.

Internal quality control (QC) procedures were performed at the beginning of each analytical run using low and high-range QC materials to monitor instrument performance. All QC values were confirmed to be within the manufacturer's specified ranges, providing assurance of the precision and accuracy of all measured hematological and biochemical parameters. Furthermore, all assay kits used for the biochemical analyses (e.g., LFTs,) were from reputable sources and were used in strict accordance with the manufacturers' instructions. This comprehensive quality control protocol ensured the integrity and reliability of all laboratory results, which is a fundamental requirement of good laboratory practice.

3.12 Method of Data Analysis

The data collected from hematological, biochemical, and histological assessments were compiled, coded, and entered into Microsoft Excel for preliminary organization. Statistical analysis was conducted using GraphPad Prism (version X.X) and SPSS (version X.X) software. Results were expressed as mean ± standard deviation (SD) for each experimental group. Comparisons between the control and treatment groups were made using one-way Analysis of Variance (ANOVA) followed by Tukey's post hoc test to determine the level of significance between group means. A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant. For hematological and biochemical parameters, numerical values obtained from laboratory assays were used directly for analysis. For histological data, qualitative assessments were made based on microscopic observations, and where applicable, semi-quantitative scoring systems were employed to evaluate the degree of histopathological changes such as

inflammation, necrosis, vascular congestion, or degenerative lesions in the liver and kidney tissues. Results were tabulated and presented using graphs, bar charts, and photomicrographs to enhance clarity and support interpretation. The analysis was structured to evaluate the dose-dependent effect of retinol on the measured parameters in pregnant Wistar rats, enabling a comprehensive comparison across all experimental groups.

3.13 Ethical Clearance

Ethical approval for this study was obtained from the Research Ethics Committee of the Kwara State Ministry of Health with the approval assigned Identification number ERC/MOH/2025/05/435. The research protocol underwent thorough review and was approved in line with national and international ethical standards governing the use of animals in scientific research. All experimental procedures involving animals were conducted in strict accordance with established institutional guidelines for the care and use of laboratory animals. Ethical clearance was granted prior to the initiation of the study, ensuring full compliance with regulatory, scientific, and welfare requirements.

3.14 Animal Welfare Compliance and Monitoring

All animal-related procedures in this study were conducted in compliance with the ethical guidelines set forth by the Kwara State Ministry of Health Ethical Committee, in accordance with national and international standards governing the care and use of laboratory animals. Ethical approval was duly obtained before the commencement of the study, and every protocol followed the principles outlined in the Guide for the Care and Use of Laboratory Animals and the Organisation for Economic Co-operation and Development framework for biomedical research. The Wistar rats were housed in clean, well-ventilated cages under standardized laboratory conditions with controlled

temperature, humidity, and a 12-hour light/dark cycle. The Wistar Rats were provided with clean drinking water and standard laboratory chow ad libitum. Daily hygiene protocols were strictly maintained to ensure a pathogen-free environment and to promote overall animal health. Throughout the study, daily welfare assessments to observe for signs of stress, pain, malnutrition, or illness. Any abnormal behavior or health complications were documented, and appropriate interventions were made according to humane endpoints. All procedures including dosing, handling, and sample collection were carried out using methods designed to minimize discomfort and stress. Pregnant rats were identified and monitored following successful mating confirmation, while non-pregnant rats were observed to ensure continued compliance with inclusion criteria. Sacrifices for histopathological and hematological analyses were carried out under proper anesthesia, following humane practices to prevent suffering. This research adhered to the 3Rs principles of animal research, Replacement, Reduction, and Refinement by using the minimum number of animals required for statistical relevance, refining techniques to lessen potential harm, and ensuring that no unnecessary duplication of experimentation occurred. Animal welfare monitoring was sustained from acquisition to sacrifice to uphold the ethical integrity of the entire experimental process.

CHAPTER FOUR

RESULTS

Introduction

The findings from the comparative study on non-pregnant and pregnant Wistar rats are detailed in this chapter. The primary objective of this research was to systematically evaluate the differential physiological and toxicological effects of varying retinol doses in both states. The animals were organized into a control group and three distinct dose groups: minimal, therapeutic, and lethal doses, to allow for a comprehensive dose-dependent analysis. The investigation involved a multi-faceted approach, with each set of parameters providing unique insights into the overall effects of the treatment. Organ weight analysis revealed a dose-dependent increase, with significant increases in liver weight observed in the minimal and lethal dose pregnant groups. While most haematological parameters showed non-significant differences, pregnant rats in the therapeutic dose group exhibited significant alterations in neutrophils, basophils, and lymphocytes. Biochemical assessments revealed specific changes in liver function, with significant alterations in albumin, total protein, and alkaline phosphatase in pregnant rats at the minimal and therapeutic dose levels. Most notably, the histopathological examination of the liver demonstrated clear and progressive dose-dependent injury, which was consistently more pronounced in pregnant rats. These results collectively highlight the differential physiological and toxicological responses to retinol between the two physiological states, with pregnancy appearing to exacerbate its effects. These findings form the basis for the detailed discussion and analysis in the subsequent sections of this chapter.

4.1 Body weight changes in wistar rats

The mean initial body weight of non-pregnant rats was significantly lower than that of pregnant animals across all groups ($p<0.05$). However, no statistically significant differences were observed in the final body weights between the groups ($p>0.05$). The data is available in Table 4.1 (Appendix I) and graphically represented in Figure 4.1.

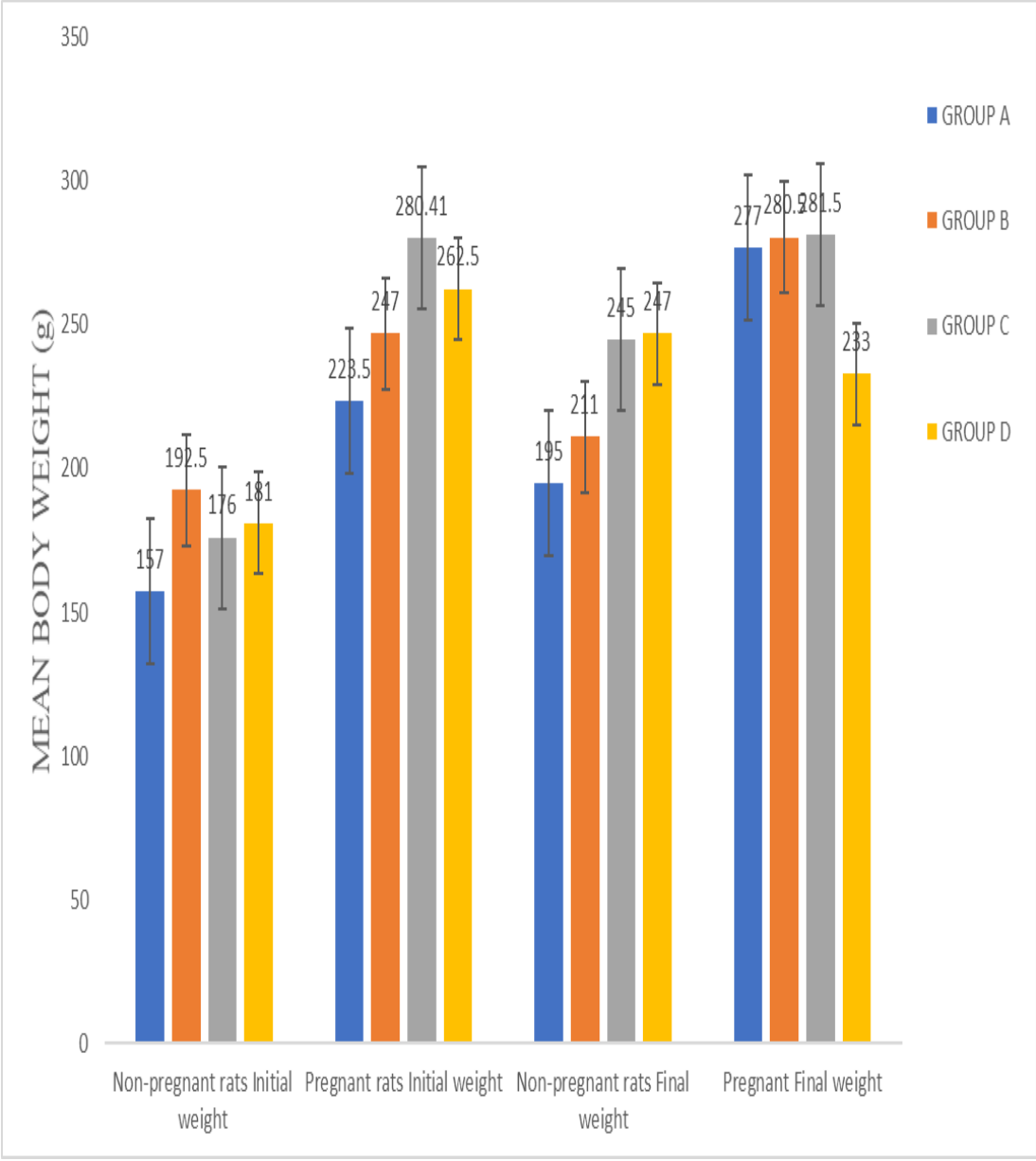

Figure 4.1 Mean body weight of the experimental animal

4.2 Organ Weight comparison between pregnant and non-pregnant wistar rats

The mean liver weight of pregnant rats was significantly higher than non-pregnant rats in the minimal (p=0.05) and lethal (p=0.04) dose groups, but not in the control (p=0.11) or therapeutic dose (p=0.15) groups. The data is available in Table 4.2 (Appendix II) and graphically represented in Figure 4.2.

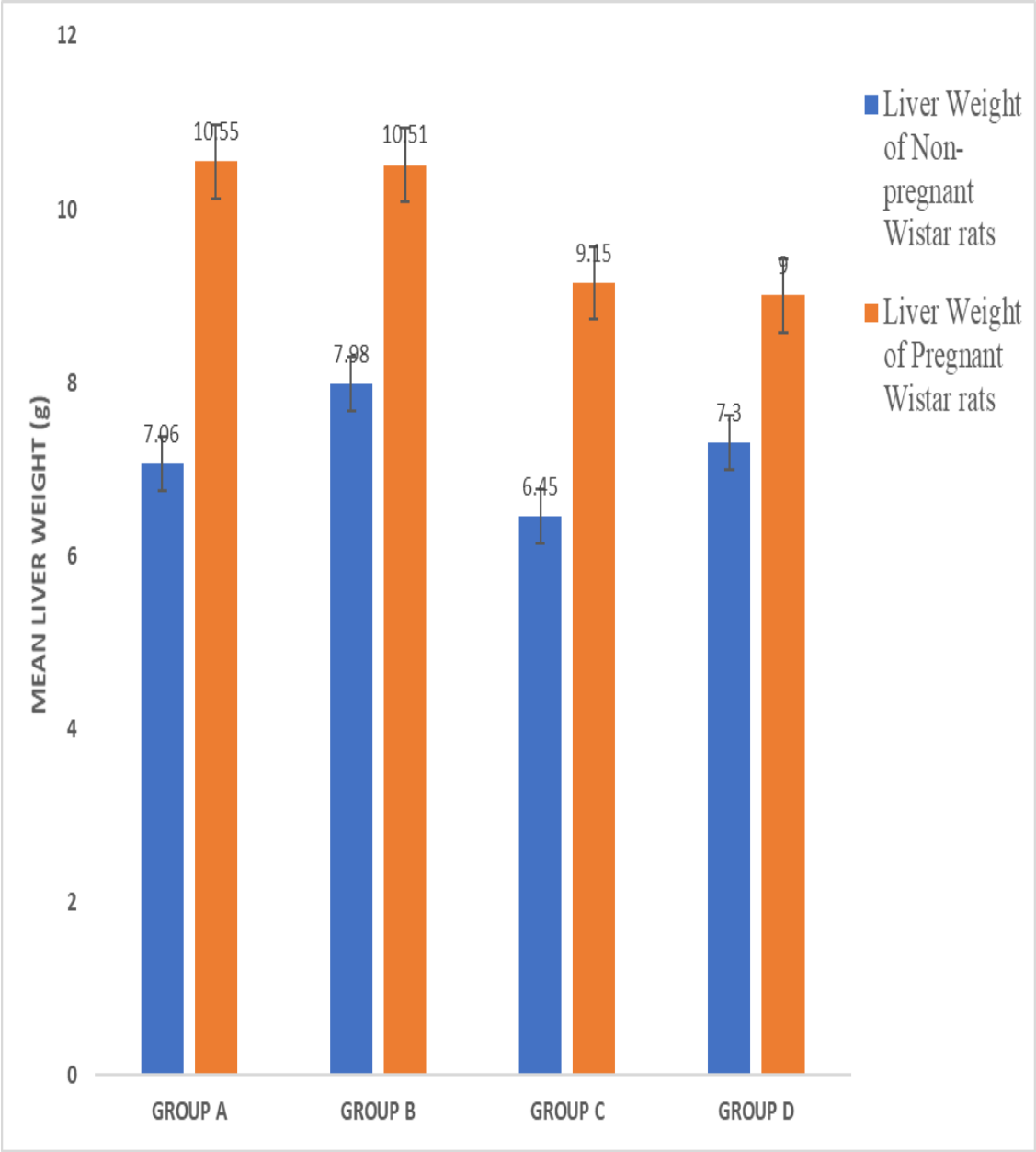

Figure 4.2: Mean liver weight of the experimental animal

4.3 Haematological parameters in pregnant and non-pregnant wistar rats

In non-pregnant rats, a statistically significant difference was found only in basophil (BASO) counts ($p<0.0001$). No significant differences were observed in any haematological parameters for the pregnant Wistar rats. The results are shown in Figure 4.3, with the corresponding data in Table 4.3 in the appendix.

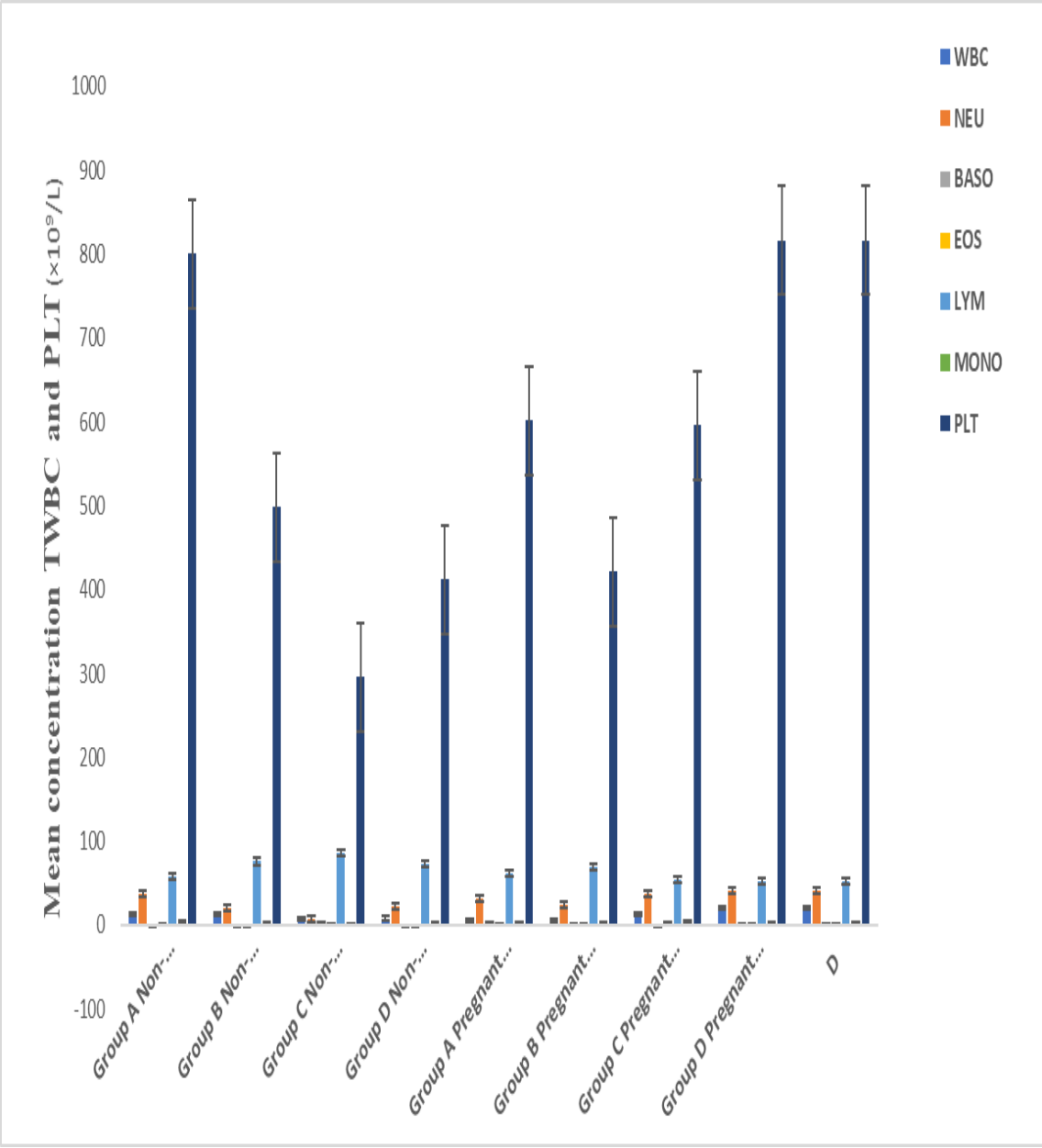

Figure 4.3 The mean concentrations of WBC and PLT parameters for pregnant and non-pregnant wistar rat

4.4 Comparision of haematological parameters between pregnant and non-pregnant wistar rats across groups

The haematological parameters of pregnant and non-pregnant Wistar rats were compared across the different experimental groups to assess the effects of the treatments. The analysis of white cell indices and platelet counts revealed a varied pattern of statistical significance. In the control group, a significant difference was found only in WBC counts ($p=0.006$), while all other parameters showed no significant differences. The data in this group suggests a baseline difference in total white blood cell count between pregnant and non-pregnant states. In the minimal dose group, no statistically significant differences were observed in any leukocyte or platelet parameters, suggesting that the minimal dose did not induce a measurable alteration in these blood cell counts in pregnant rats compared to their non-pregnant counterparts. A different pattern emerged in the therapeutic dose group, with significant differences observed in neutrophils ($p=0.03$), basophils ($p=0.007$), and lymphocytes ($p=0.02$). These findings indicate that the therapeutic dose significantly altered these specific blood cell populations in the pregnant state. In contrast, no significant differences were found in WBC, EOS, MONO, or PLT. Finally, no statistically significant differences were observed across all tested haematological parameters in the lethal dose group. This suggests that the physiological response in this group did not manifest as a clear statistical difference in blood cell counts between the pregnant and non-pregnant rats. The complete results for all groups are illustrated in Chart 4.4 (a-b), with the corresponding data presented in Table 4.4 in the appendix.

Figure 4.4a The comparison of white blood cell and platelet parameters between pregnant and non-pregnant rats in the control group showed a significant difference only in the total white blood cell count (WBC).

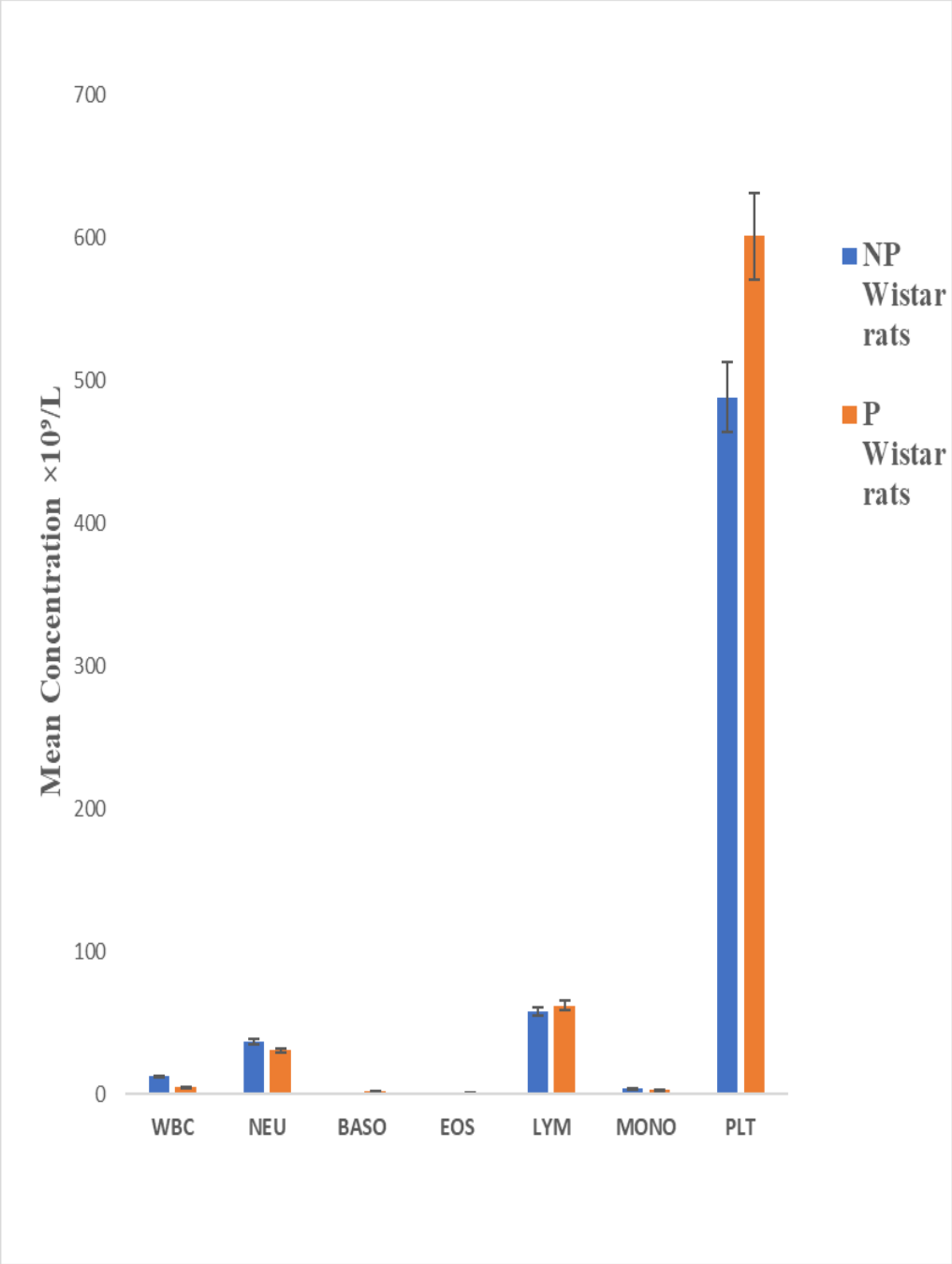

Figure 4.4a: Haematological parameters of control dose group A

Figure 4.4b In the minimal dose group, no statistically significant differences were found across any of the white blood cell and platelet parameters between pregnant and non-pregnant rats.

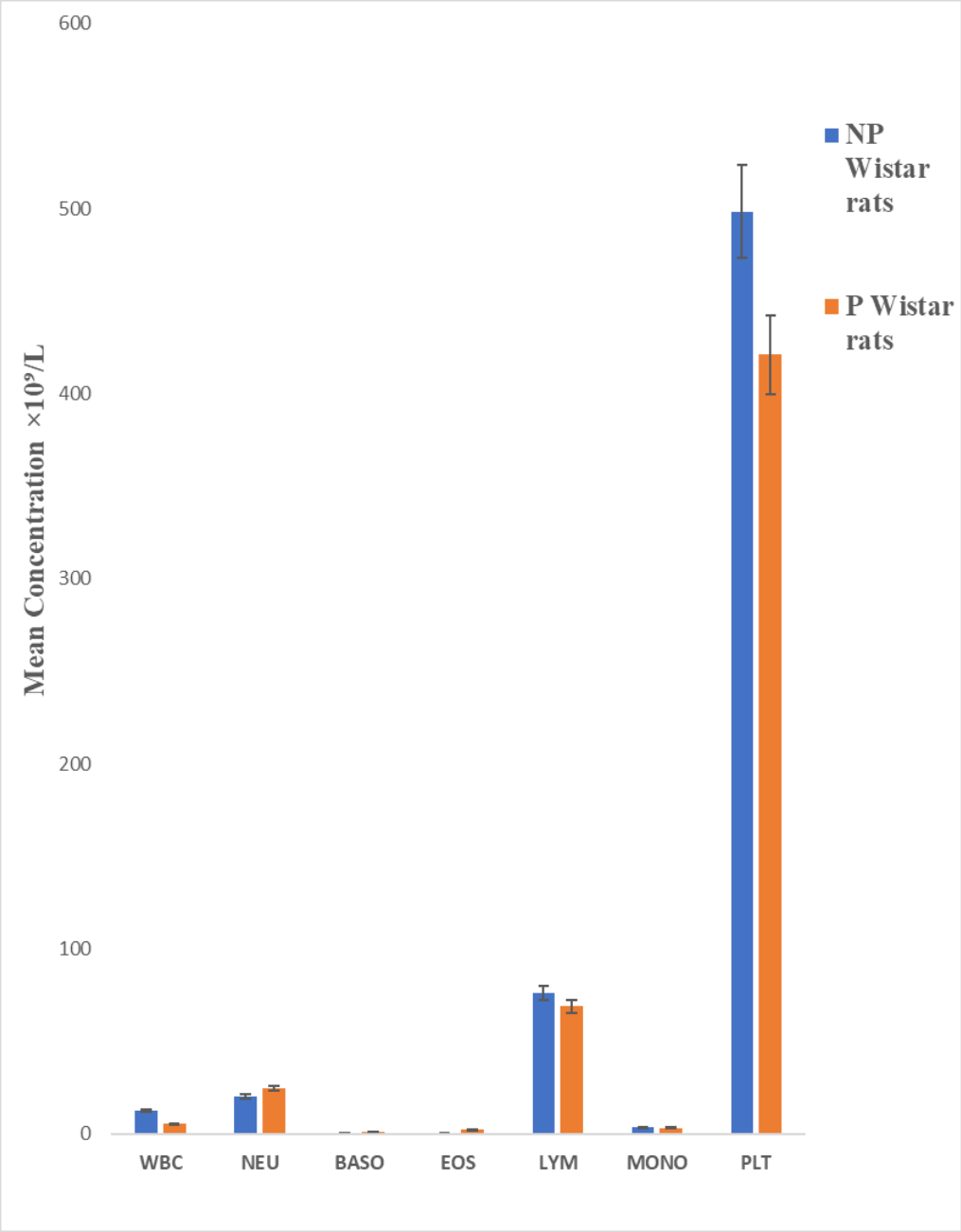

Figure 4.4b: Haematological parameters of minimal dose group B

Figure 4.4c In the therapeutic dose group, significant alterations were found in neutrophil (NEU), basophil (BASO), and lymphocyte (LYM) counts when comparing pregnant and non-pregnant rats.

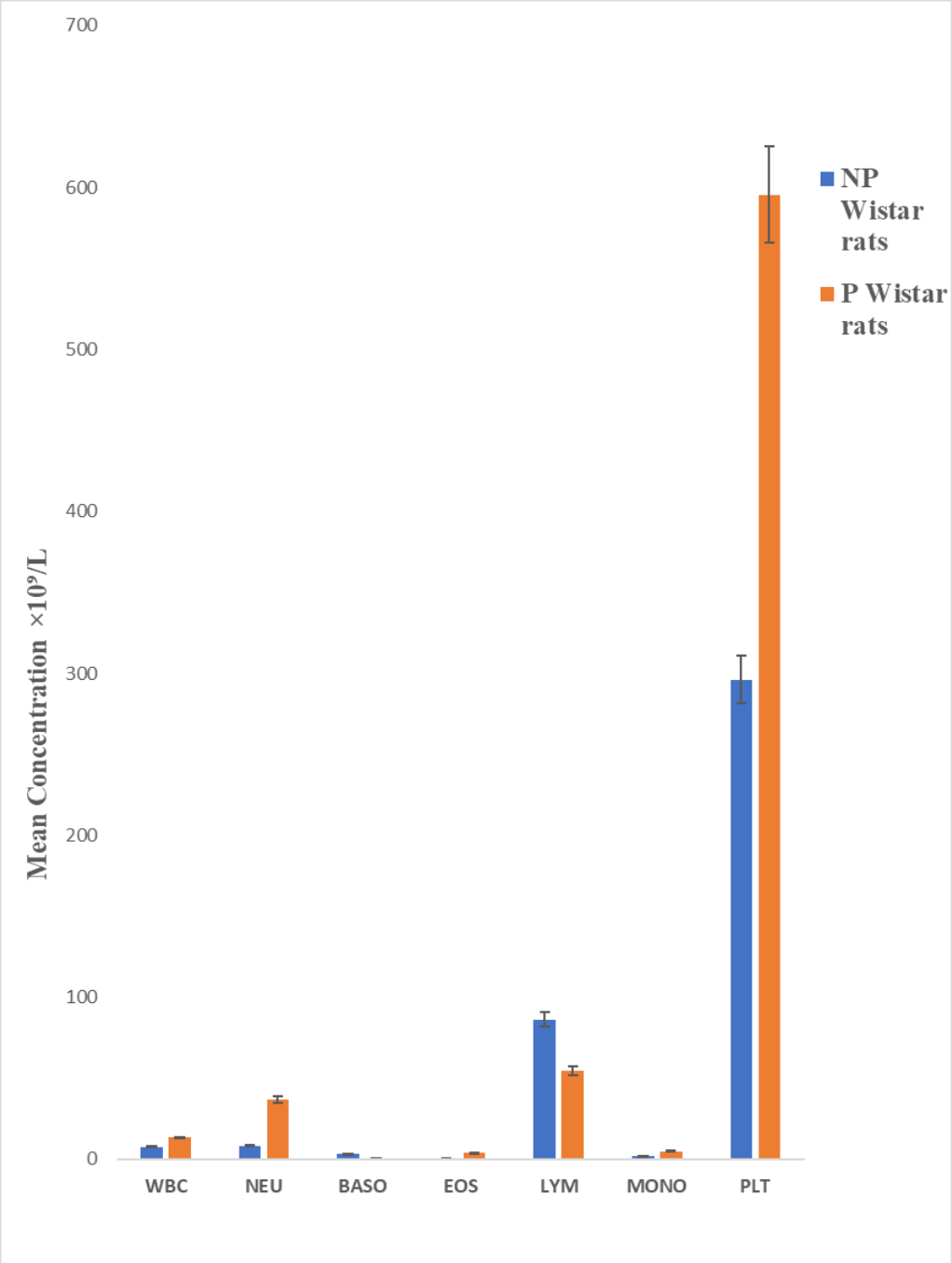

Figure 4.4c: Haematological parameters of therapeutic dose group C

Figure 4.4d The comparison of white blood cell and platelet parameters in the lethal dose group showed no statistically significant differences in any of the measured parameters between pregnant and non-pregnant rats.

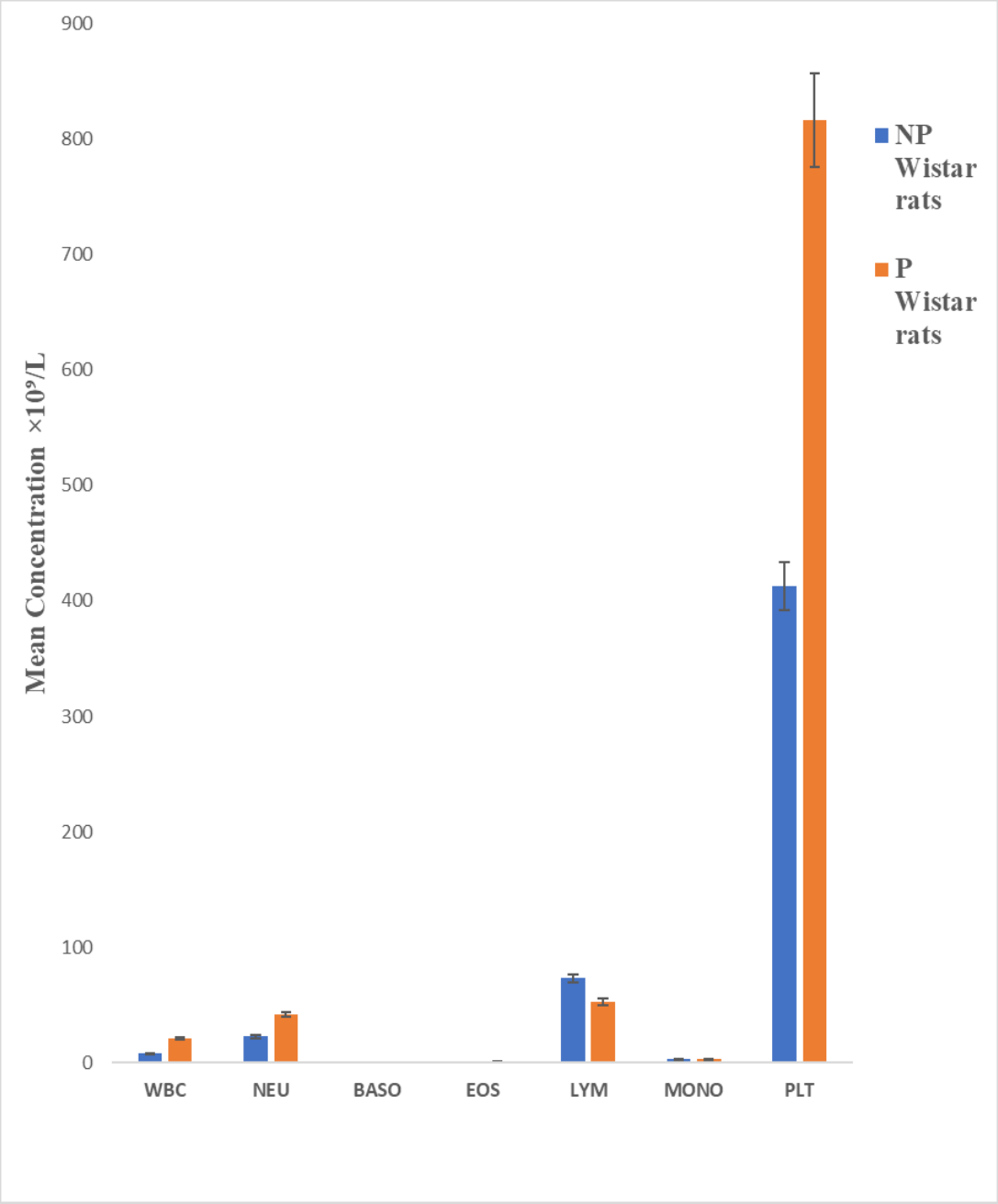

Figure 4.4d: Haematological parameters of lethal / toxic group D

4.5 Biochemical Parameters in Pregnant and Non-Pregnant Wistar Rats

For both non-pregnant and pregnant Wistar rats, no significant differences were found in any hepatic or protein markers across the groups. The p-values for all parameters (total protein, albumin, ALT, AST, ALP, T.Bil, and D.Bil) were non-significant. These findings are presented in Figure 4.5, with the data in Table 4.5 in the appendix.

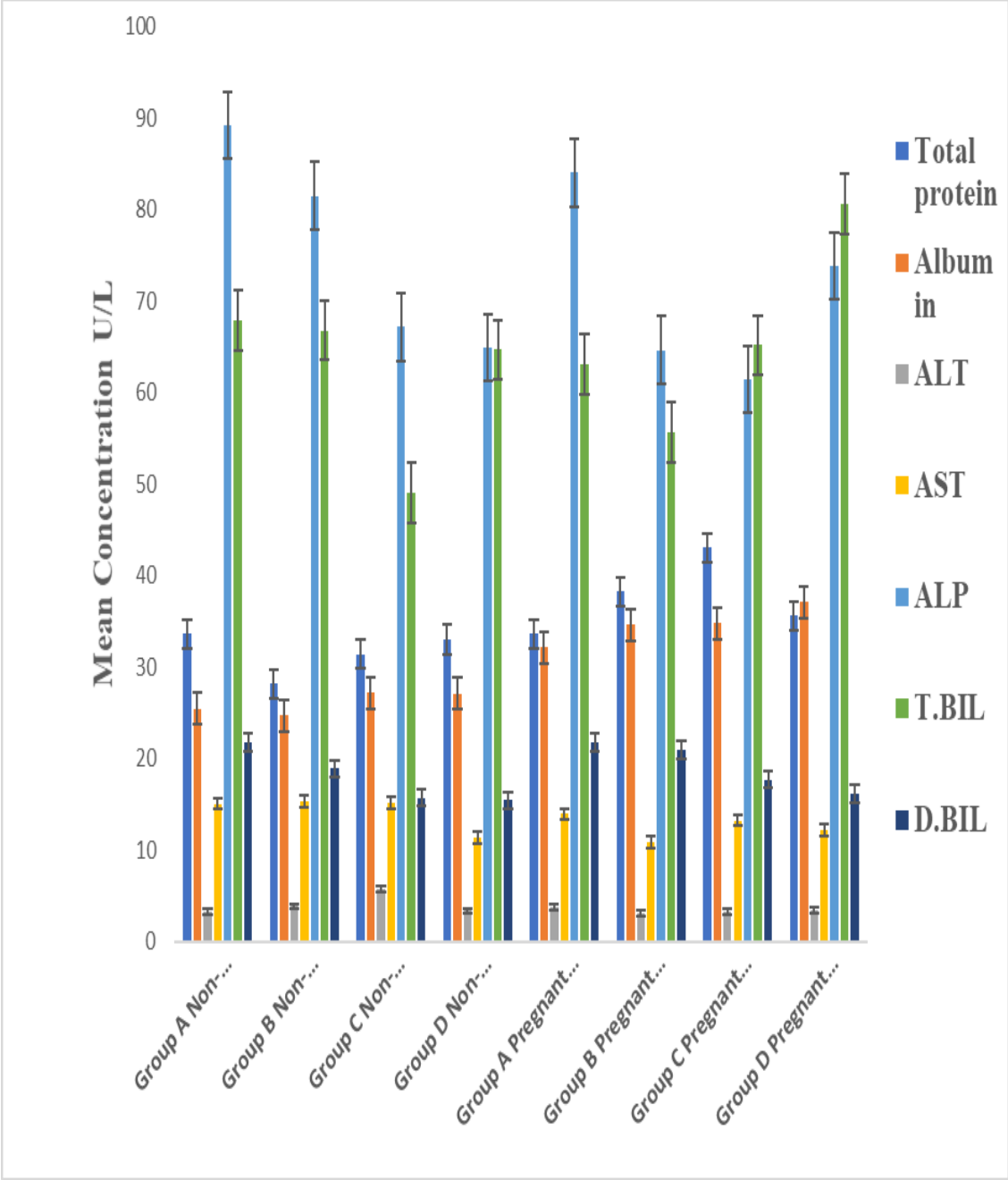

Figure 4.5: Mean concentration of biochemical parameters

4.6 Comparison of Biochemical Parameters Between Pregnant and Non-Pregnant Wistar Rats Across Groups

The liver function parameters of pregnant and non-pregnant Wistar rats were compared across all experimental groups to determine the effects of retinol administration. The analysis focused on key markers including albumin, total protein, ALT, AST, ALP, total bilirubin (T.Bil), and direct bilirubin (D.Bil). This comprehensive assessment provided critical insights into the liver's functional state. In the control group (A), no statistically significant differences were observed between pregnant and non-pregnant rats for any of the tested parameters. While some mean concentrations were higher (albumin, ALT, AST) or lower (ALP, T.Bil, D.Bil) in pregnant rats, these differences were not significant (all p-values were greater than 0.05). In the minimal dose group (B), significant differences emerged. Pregnant rats showed a significant increase in albumin ($p=0.02$) and total protein ($p=0.05$), while a significant decrease was noted in ALP ($p=0.05$). No significant differences were found in the other parameters, including ALT ($p=0.60$), AST ($p=0.28$), T.Bil ($p=0.68$), and D.Bil ($p=0.12$). In the therapeutic dose group (C), the only statistically significant finding was a higher concentration of total protein in pregnant rats ($p=0.03$). All other parameters—albumin ($p=0.43$), ALT ($p=0.53$), AST ($p=0.28$), ALP ($p=0.63$), T.Bil ($p=0.19$), and D.Bil ($p=0.24$) showed no significant differences. In the lethal dose group (D), no statistically significant differences were observed between pregnant and non-pregnant rats for any of the measured liver function parameters. These varying results across groups highlight the complex and dose-dependent nature of retinol's effects on liver function. All p-values were greater than 0.05. The complete data for these findings is presented in Table 4.6 in the appendix, and a visual representation is shown in Figure 4.6 (a-b).

Figure 4.6a In the control group, the comparison of liver function parameters found no statistically significant differences between pregnant and non-pregnant rats.

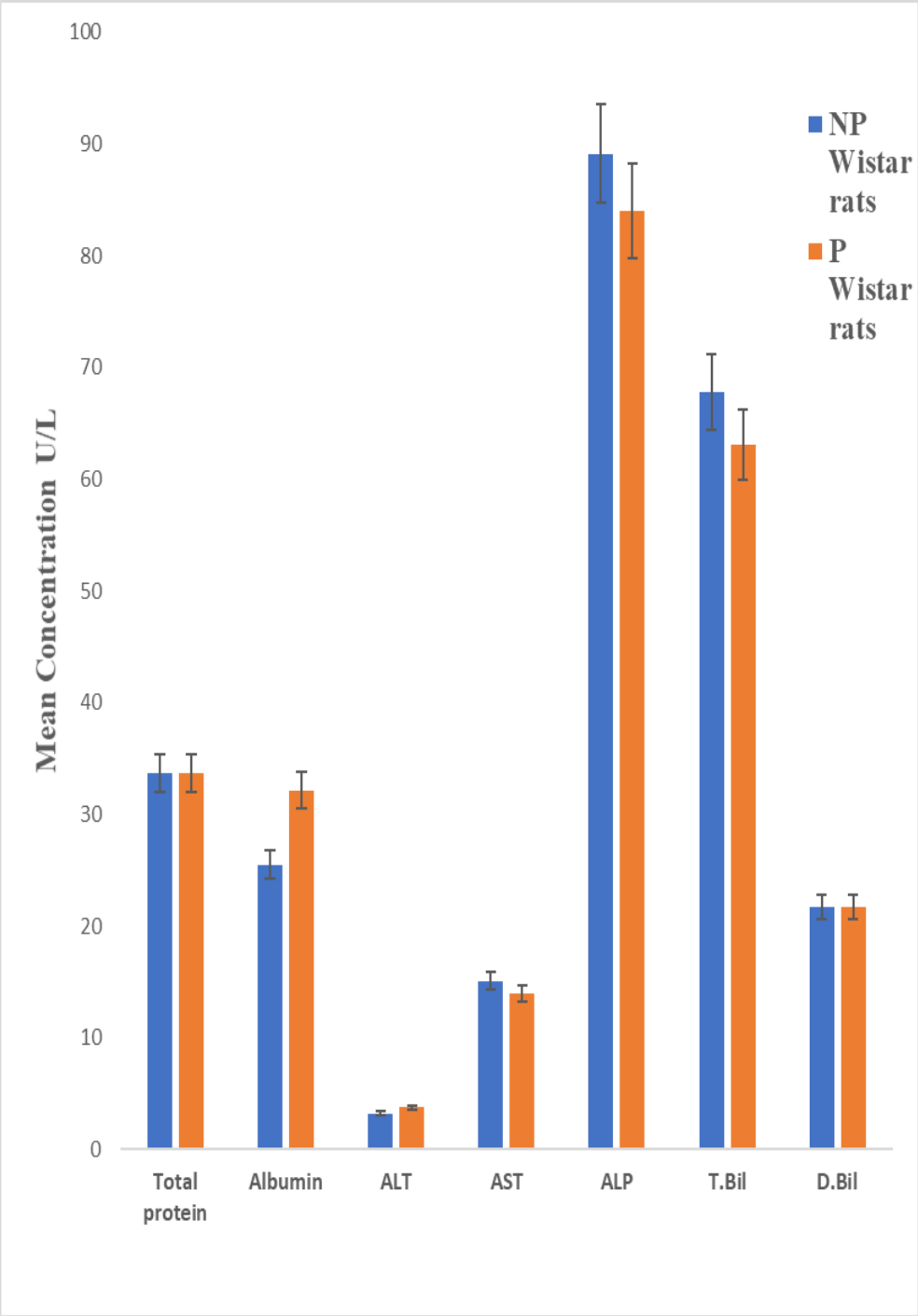

Figure 4.6a: Biochemical parameters of control group A animals

Figure 4.6b In the minimal dose group, pregnant rats had significantly higher albumin and total protein, and significantly lower ALP compared to non-pregnant rats.

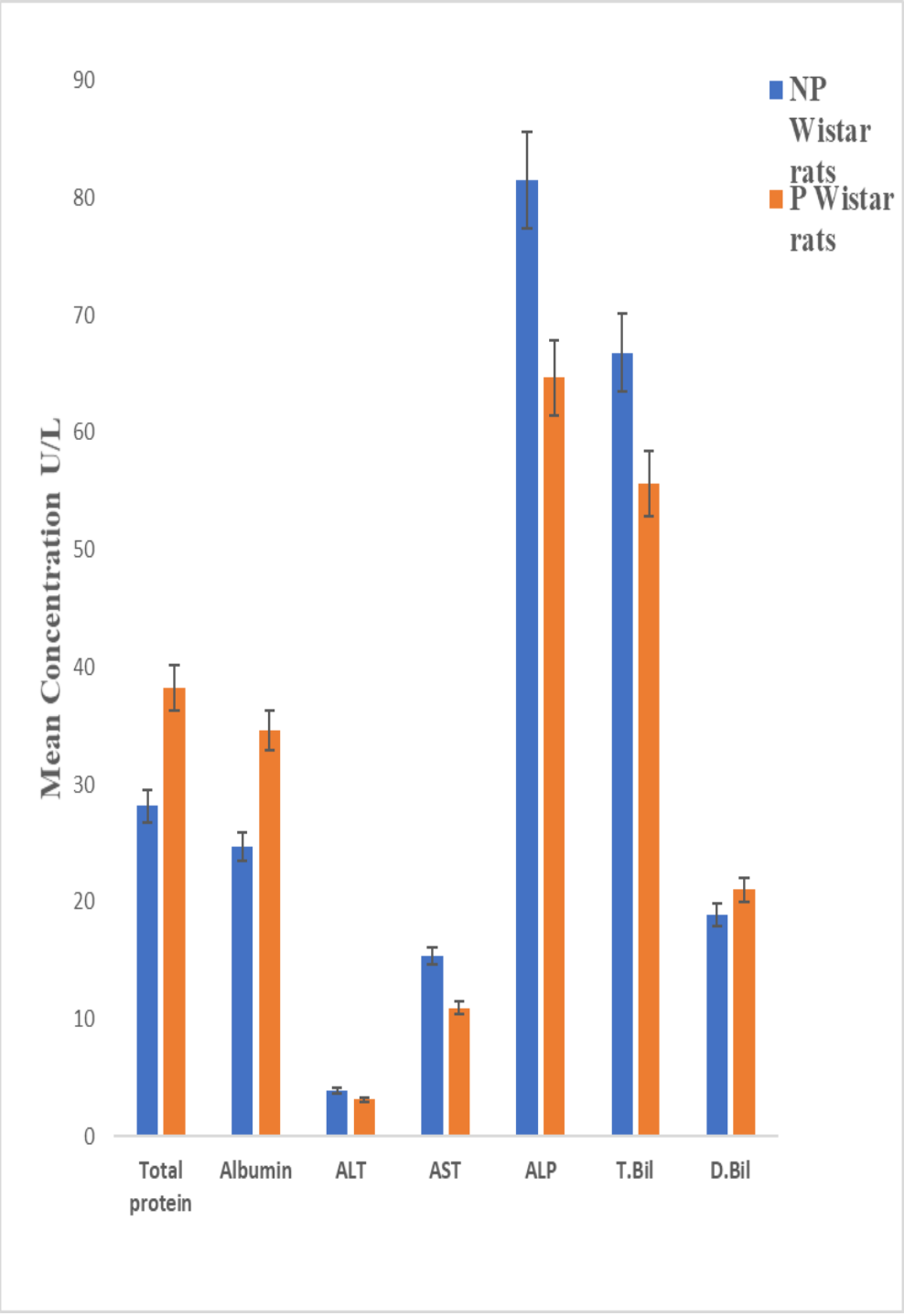

Figure 4.6b Biochemical parameters of minimal dose group B animals

Figure 4.6c: For the therapeutic dose group, the comparison revealed a statistically significant increase in total protein in pregnant rats, while other parameters showed no significant differences.

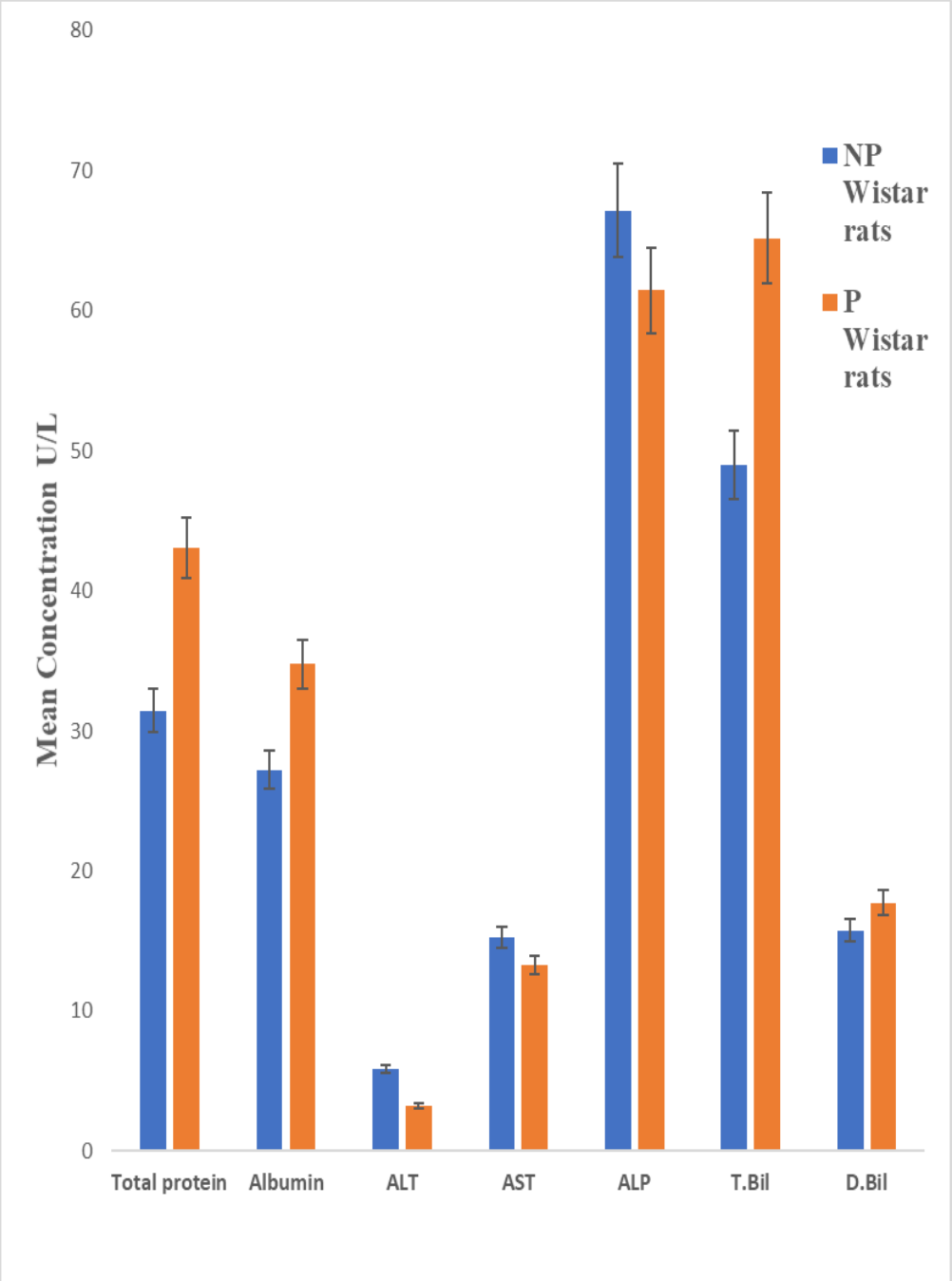

Figure 4.6c: Biochemical parameters of therapeutic dose group C animals

Figure 4.6d: In the lethal dose group, no statistically significant differences were observed across any of the liver function parameters when comparing pregnant and non-pregnant rats.

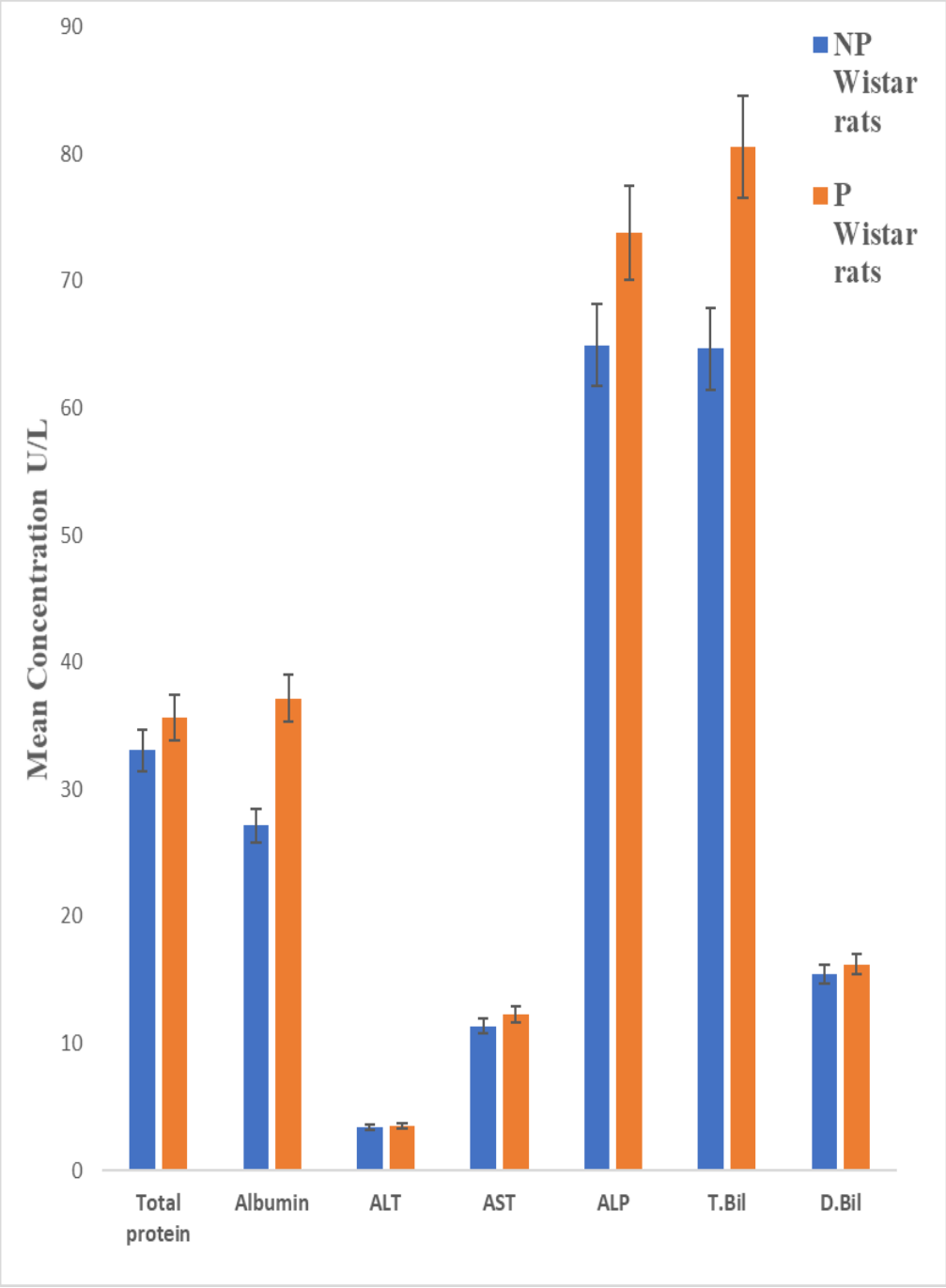

Figure 4.6d: Biochemical parameters of lethal / toxic group D animals

4.7 Liver histomorphology of non-pregnant wistar rats across experimental groups

The liver histology of non-pregnant rats revealed progressive but less severe alterations compared to the pregnant group. In the Control Group (A), the liver appeared entirely normal, showing well-arranged hepatocyte cords, intact sinusoidal channels, and resident Kupffer cells in their usual positions. No degenerative, necrotic, or inflammatory changes were observed, confirming healthy hepatic morphology. In the Minimal Dose Group (B), early degenerative changes were evident. The photomicrographs revealed compact nests of hepatocytes with peri-nuclear halos and mild cytoplasmic granulation. Occasional necrotic foci and scattered inflammatory cells were also present, suggesting the onset of retinol-induced toxicity at low dosage. In the Therapeutic Dose Group (C), more advanced alterations appeared, with hepatic oedema leading to separation of hepatocyte cords and dilation of sinusoidal spaces. Multiple peri-nuclear halos, limited areas of necrosis, and appreciable infiltration of inflammatory cells were observed, indicating tissue stress and functional compromise. Finally, in the Lethal Dose Group (D), severe hepatocellular injury was observed. The liver displayed extensive feathering of hepatocytes with abundant peri-nuclear halos, widespread necrosis, and dense inflammatory cell infiltration, confirming significant hepatotoxicity. These findings establish that high-dose retinol induces progressive liver injury in the non-pregnant state, although the extent of damage is less pronounced than in pregnancy. The full details of these changes are illustrated in Figure 4.8 (A–D).

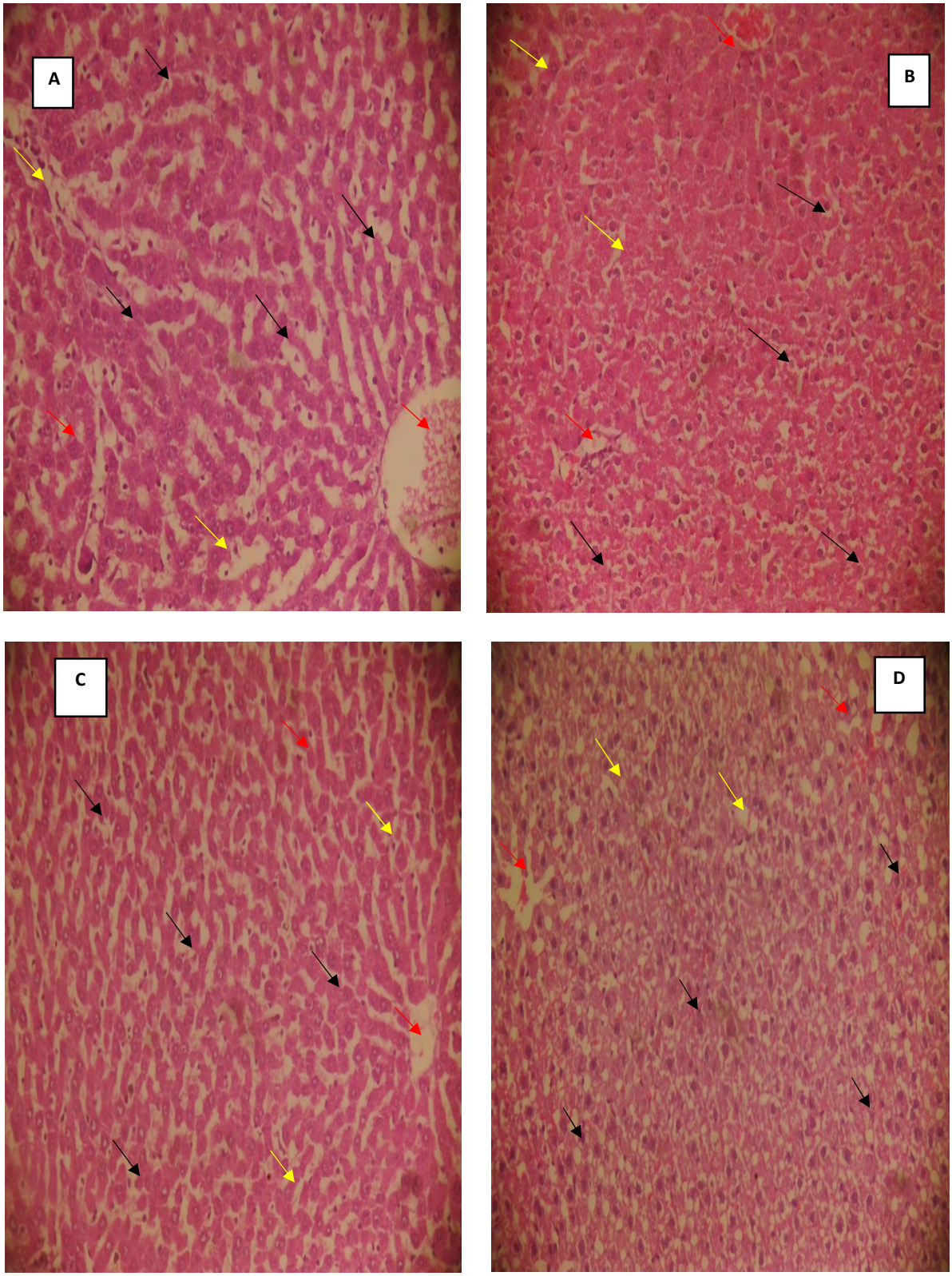

Figure 4.7: Photomicrograph of the liver morphology of non- pregnant Wistar rats from group A-D

Figure 4.8: Liver Morphology of Pregnant Wistar Rats across experimental groups

The liver histology of pregnant rats revealed a progressive spectrum of changes with increasing retinol dosage, which appeared more severe than in the non-pregnant state. In the Control Group (A), the overall hepatic architecture was preserved, with well-arranged hepatocyte cords, intact sinusoids, and Kupffer cells in their normal positions. Subtle reactive features were observed, including mild anisonucleosis and occasional peri-nuclear halos, while minimal necrotic foci and scattered inflammatory cells appeared within the hepatic channels, suggesting pregnancy-associated hepatic stress even without treatment. In the Minimal Dose Group (B), the changes became more pronounced, with irregularly arranged hepatocytes forming nests, multiple peri-nuclear halos, cytoplasmic granulation, and early evidence of hepatocyte feathering. Focal necrosis was evident though not widespread, while inflammatory cell infiltration remained limited. In the Therapeutic Dose Group (C), advanced hepatocellular injury was observed, with hepatic oedema separating hepatocyte cords, sinusoidal dilation, and large areas of feathered hepatocytes. Numerous peri-nuclear halos, more extensive necrosis, and clear infiltration of inflammatory cells were present, along with early cholestatic features, indicating disruption of liver function. Finally, in the Lethal Dose Group (D), the liver exhibited the most severe pathology, characterized by massive hepatocyte feathering, abundant peri-nuclear halos, widespread necrosis, dense inflammatory cell infiltration, and significant architectural collapse. Collectively, these findings confirm that retinol induces more severe hepatotoxicity in pregnancy, with progressive damage evident from the control to lethal dose groups, as illustrated in Figure 4.7 (A–D).

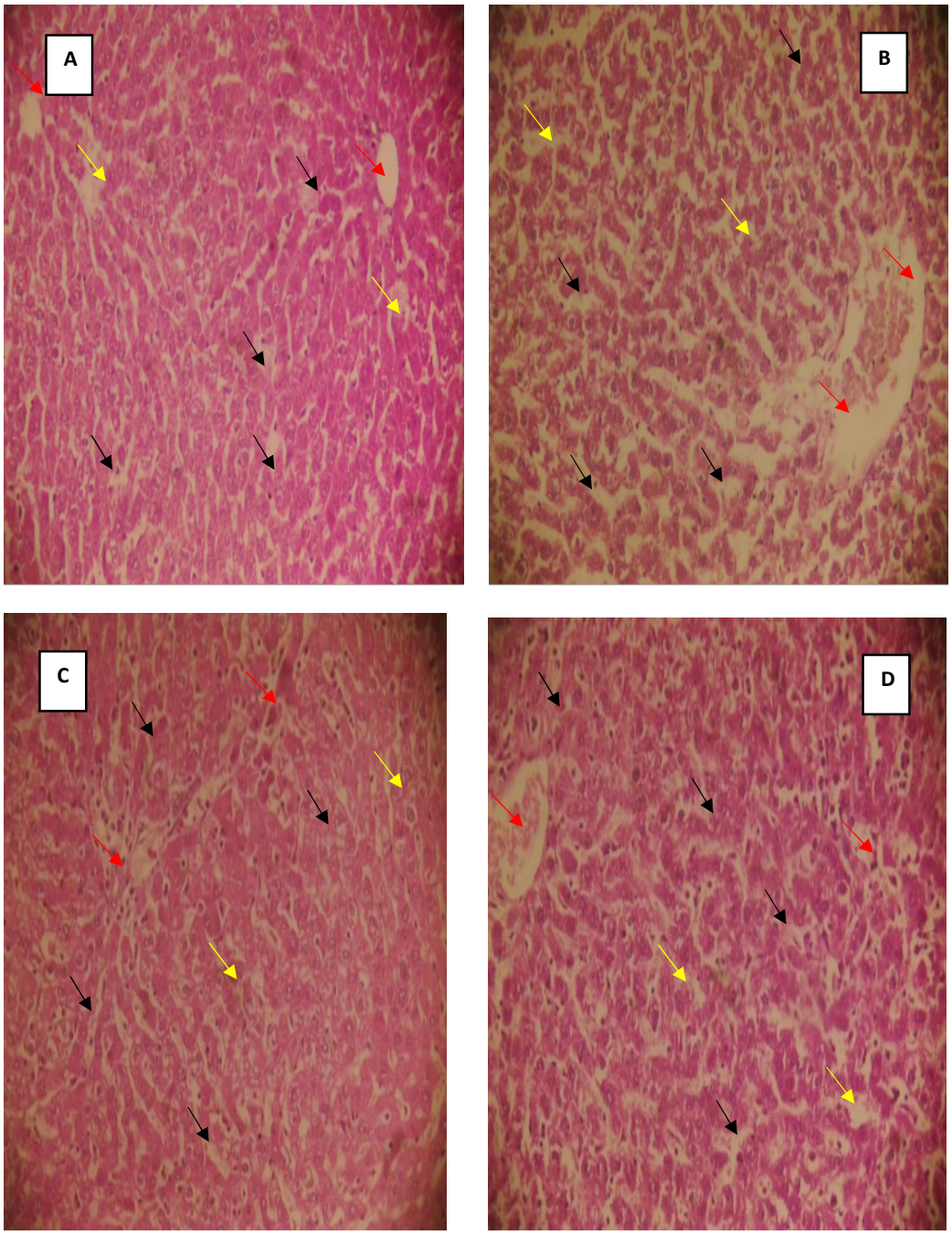

Figure 4.7: Photomicrograph of the liver morphology of non- pregnant Wistar rats from group A-D

CHAPTER FIVE

DISCUSSION

Discussion

Retinol is an essential micronutrient involved in numerous physiological processes, including epithelial integrity and hematopoiesis. However, both deficiency and excess can lead to adverse health outcomes (Patili *et al.,* 2023). This study investigated the effects of graded doses of retinol on liver function, hematological parameters, and liver histology in pregnant and non-pregnant Wistar rats.

There was a significant increase in final body weight across all groups, except for pregnant rats administered the lethal dose of retinol, who showed significant weight loss. This weight reduction may indicate retinol toxicity, consistent with earlier findings that high doses of vitamin A can result in tissue damage, decreased appetite, and impaired nutrient absorption (Ayeleso *et al.,* 2019; Penniston and Tanumihardjo, 2021). In contrast, minimal and therapeutic doses appeared to support normal physiological growth, aligning with vitamin A's known role in immune function and cell differentiation (Tanumihardjo, 2021).

Liver weight increased significantly in pregnant Wistar rats in the minimal and lethal dose groups, suggesting physiological and pathological hepatic enlargement, respectively. While the minimal dose-induced liver enlargement could be attributed to pregnancy-associated metabolic demands (Woods *et al.,* 2020), the liver hypertrophy in the lethal dose group is more likely due to hepatotoxic effects of high retinol intake. Retinol toxicity is known to induce hepatic cell hypertrophy, congestion, and fatty infiltration (Penniston and Tanumihardjo, 2021).

Regarding hematological indices, there was no significant difference in most parameters in non-pregnant rats, except for basophils, whose elevated levels may

indicate a mild inflammatory or hypersensitivity response. Among pregnant rats, a significant increase in hemoglobin levels was observed with increasing retinol doses. This may reflect retinol-enhanced erythropoiesis, as vitamin A improves iron mobilization and red cell production (Ramakrishnan *et al.,* 2022). In the therapeutic dose group, pregnant rats showed significant increases in RBC, NEU, HB, and HCT compared to non-pregnant counterparts, suggesting possible erythropoietic stimulation due to retinoid-induced oxidative stress. Simultaneously, lymphocyte and basophil counts were significantly lower, potentially indicating immunomodulatory effects or suppression associated with higher retinol levels (Villamor and Fawzi, 2020; Stephensen, 2021).

Biochemical liver function parameters (ALT, AST, ALP, total and direct bilirubin, total protein, albumin) remained largely unchanged across groups, except for minor variations in albumin and total protein. Pregnant rats in the minimal and therapeutic dose groups showed elevated albumin and total protein levels compared to non-pregnant rats. These differences may reflect increased hepatic protein synthesis stimulated by retinol, countering the typical hemodilution seen in pregnancy (Pavord *et al.,* 2022). The absence of significant changes in liver enzymes or bilirubin across most groups suggests that early-stage retinol toxicity may not always be reflected in conventional liver function tests, especially over short exposure periods.

Histological analysis of liver tissues confirmed structural alterations associated with retinol dosing. Control liver samples exhibited normal architecture, while minimal dose groups displayed early degenerative changes such as peri-nuclear halos and granular cytoplasm. Therapeutic and lethal doses resulted in more severe changes, including hepatocyte feathering, necrosis, and signs of cholestasis. These findings are consistent with previous studies documenting retinoid-induced hepatic injury due to

oxidative stress and disrupted lipid metabolism (Rombaldi *et al.,* 2018; Kullak-Ublick, 2017; Biesalski, 2020). Taken together, the study demonstrates that while minimal and therapeutic retinol doses may support physiological function, excessive intake, particularly during pregnancy, poses significant risks to hepatic and hematologic health. These effects are more pronounced in pregnant rats, likely due to altered drug metabolism, increased oxidative stress, and higher nutrient demands during gestation.

Retinol (vitamin A) is an essential micronutrient involved in various physiological processes, including epithelial maintenance, hematopoiesis, immune modulation, and cellular differentiation. However, both hypovitaminosis and hypervitaminosis A are known to cause systemic dysfunctions (Patili *et al.,* 2023). This study assessed the dose-dependent effects of retinol on liver function, hematological parameters, and hepatic histology in pregnant and non-pregnant Wistar rats, revealing patterns consistent with and divergent from prior findings in animal and human models.

The study observed a significant increase in final body weight across most groups, except for pregnant rats exposed to lethal doses of retinol, who experienced notable weight loss. This outcome is congruent with earlier studies by Markou *et al.* (2021) and Fox *et al*. (2020), who reported that hypervitaminosis A leads to reduced appetite, gastrointestinal distress, and subsequent weight loss due to hepatotoxicity and impaired nutrient metabolism. In contrast, the weight gain seen in rats administered minimal and therapeutic doses aligns with findings by Awasthi and Awasthi (2020), emphasizing vitamin A's role in supporting growth, immunity, and tissue repair. Similar physiological improvements following low-dose retinoid supplementation have been documented in pregnant rodent models by Pfau *et al.* (2023), further validating this study's findings.

Liver weight significantly increased in pregnant rats receiving minimal and lethal retinol doses, suggesting dual physiological and pathological hypertrophy. While hepatic enlargement during pregnancy can be attributed to increased metabolic demands (Woods *et al.,* 2020), the exaggerated liver size in the lethal dose group likely reflects hepatotoxicity, a condition similarly reported by Penniston and Tanumihardjo, (2006) who observed hepatic steatosis, fibrosis, and congestion in rats following excessive retinol intake. Study by Shmarakov *et al.* (2013) argued that the liver has a high retinoid storage capacity and can buffer against short-term overload, this study's histopathological data contradict that, showing visible tissue damage even over a limited exposure period, particularly in pregnant subjects.

Regarding hematological indices, non-pregnant rats exhibited relatively stable values, except for increased basophil counts, which may signify a mild inflammatory or hypersensitivity response, consistent with observations by Jyothi *et al.* (2024) in retinol-treated rodents. More pronounced hematological changes were recorded in pregnant rats, particularly increased hemoglobin, RBCs, neutrophils, and hematocrit levels at therapeutic doses. These trends are in line with Silva *et al*. (2021), who demonstrated that retinol supports erythropoiesis by enhancing iron metabolism and erythroid precursor cell proliferation. Moreover, reductions in lymphocyte and basophil counts suggest an immunosuppressive shift, echoing findings by Jimenez *et al.* (2010), who suggested that excess vitamin A modulates cytokine expression and suppresses lymphocyte proliferation, particularly during pregnancy.

Liver function test results were largely within normal ranges, with minor elevations in albumin and total protein levels among pregnant rats administered minimal and therapeutic doses. These increases may indicate enhanced hepatic synthetic activity stimulated by retinol, as previously proposed by Tacke *et al.,* (2018). Although,

despite histological signs of hepatotoxicity in high-dose groups, conventional liver enzymes (ALT, AST, ALP) remained unchanged, a pattern similarly observed in Mahdieh *et al.* (2012), who noted that biochemical markers might not always reflect early-stage hepatic injury, especially in the context of adaptive physiological changes during gestation.

Histopathological evaluation confirmed retinol-induced hepatic alterations. While control livers maintained normal architecture, minimal-dose groups exhibited early degenerative changes such as peri-nuclear halos, possibly due to mild oxidative stress. More severe damage including necrosis, cytoplasmic feathering, and cholestasis was evident in therapeutic and lethal dose groups. These findings closely support those of Yohei *et al.* (2015), who highlighted hepatocellular vulnerability to oxidative damage and lipid peroxidation following excessive retinoid exposure. According to study by Chen *et al.* (2025) it was noted that even sub-lethal retinol levels can disrupt hepatic microarchitecture through mitochondrial dysfunction, lending credence to the degenerative changes seen in this study. This study found that pregnant rats were more susceptible to retinol-induced damage, which may reflect increased metabolic demands, altered retinoid pharmacokinetics, and reduced antioxidant defenses during gestation. This observation supports the cautionary stance of Ross *et al.* (2013), who emphasized the teratogenic and toxic risks of vitamin A excess during pregnancy.

Conclusion

This study evaluated the effects of varying doses of retinol on liver function, haematological indices responses in pregnant and non-pregnant Wistar rats. The findings revealed that while minimal and therapeutic doses of retinol support normal physiological functions, excessive (lethal) doses result in significant hepatotoxic and hematological alterations, particularly in pregnant rats.

Retinol at minimal and therapeutic doses did not significantly impair liver function or haematological parameters, indicating a margin of safety within these ranges. However, the administration of lethal doses was associated with increased liver weight and histological evidence of hepatic degeneration and necrosis, especially among pregnant rats, highlighting the increased susceptibility of the maternal liver to retinoid-induced toxicity. Moreover, retinol appeared to influence erythropoiesis, as evidenced by increased hemoglobin levels, and altered certain white blood cell parameters, suggesting a dose-dependent impact on the hematopoietic and immune systems.

Although no significant changes were observed in routine liver function test markers such as ALT, AST, ALP, or bilirubin at lower doses, high-dose retinol exposure may induce subclinical hepatic stress not captured by standard biochemical assays. This shows the importance of integrating histological evaluation with biochemical assessments in toxicity studies. While retinol is essential for normal growth and hematopoiesis, its administration, particularly at high doses, should be carefully monitored during pregnancy due to the risk of hepatotoxicity and haematological disturbances.

Recommendations

In relation to the findings of the present study the followings are recommended;

1. Healthcare providers should exercise caution when prescribing retinol or vitamin A derivatives to pregnant women, considering potential risks to liver health.

2. Exploration of safer alternatives with minimal hepatic and renal toxicity during pregnancy should be prioritized.

3. Public Awareness: Increase awareness of potential risks of high-dose vitamin A intake during pregnancy through patient education and public health policies.

4. Future studies should assess the effect of duration of exposure to different doses of retinol intake on the liver.

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APPENDIX I

Table 4.1: The mean weight of the Wistar rats in relation to their groups

Group	Non-pregnant rats' Initial weight	Pregnant rats' Initial weight	*p*-value	Non-pregnant rats' Final weight	Pregnant Final weight	*p*-value
A	157.00 ± 4.24	223.50 ± 7.78	0.009	195.00 ± 32.53	277.00 ± 2.83	0.071
B	192.50 ± 0.71	247.00 ± 8.49	0.012	211.00 ± 14.14	280.50 ± 20.51	0.059
C	176.00 ± 4.24	280.41 ± 16.97	0.014	245.00 ± 62.23	281.50 ± 24.75	0.521
D	181.00 ± 5.66	262.50 ± 16.26	0.022	247.00 ± 59.40	233.00 ± 0.00	0.771

Level of significance $p \leq 0.05$

APPENDIX II

Table 4.2: The mean liver and kidney weight between pregnant and non-pregnant Wistar rats.

Liver	Non-pregnant Wistar rats	Pregnant Wistar rats	*p*-value	Kidney	Non-pregnant Wistar rats	Pregnant Wistar rats	*p*-value
A	7.06 ± 1.08	10.55 ± 1.46	0.11	**A**	1.11 ± 0.06	1.37 ± 0.09	0.07
B	7.98 ± 0.38	10.51 ± 0.73	0.05	**B**	1.22 ± 0.02	1.40 ± 0.16	0.26
C	6.45 ± 0.01	9.15 ± 1.66	0.15	**C**	1.11 ± 0.01	1.55 ± 0.06	0.01
D	7.30 ± 0.52	9.00 ± 0.01	0.04	**D**	1.12 ± 0.02	1.35 ± 0.00	0.004

Level of significance $p \leq 0.05$

APPENDIX III

Table 4.3: The mean concentration of haematological parameters among

Parameters	Non-pregnant Wistar rats					Pregnant Wistar rats				
Group	**A**	**B**	**C**	**D**	***p*-value**	**A**	**B**	**C**	**D**	***p*-value**
WBC	12.59 ± 0.21	12.73 ± 0.05	7.46 ± 1.32	8.24 ± 5.86	0.30	5.34 ± 2.52	5.52 ± 2.08	13.15 ± 5.52	20.86 ± 6.80	0.08
NEU	3680 ± 18.95	20.10 ± 9.76	8.15 ± 0.35	22.75 ± 0.78	0.21	31.05 ±34.15	24.55 ±18.37	36.75 ± 7.43	41.60 ± 6.51	0.84
BASO	0.25 ± 0.07	0.30 ± 0.14	3.40 ± 0.28	0.25 ± 0.21	<0.001	2.55 ± 3.18	1.05 ± 0.92	0.35 ± 0.21	0.40 ±0.14	0.57
EOS	0.50 ± 0.56	0.3500 ± 0.07	0.75 ± 0.64	0.30 ± 0.28	0.76	1.10 ± 0.99	2.25 ± 2.76	3.60 ± 3.25	1.80 ± 1.56	0.75

pregnant and non-pregnant Wistar rats in relation to their experimental groups.

LYM	58.15 ± 20.15	75.85 ± 9.55	86.10 ± 1.13	73.30 ± 2.55	0.24	62.45 ± 33.87	68.90 ± 23.90	54.45 ± 6.86	52.90 ± 7.07	0.86
MONO	4.45 ± 0.50	3.40 ± 0.14	1.60 ± 1.13	3.40 ± 1.27	0.13	2.85 ± 1.91	3.25 ± 1.91	4.85 ± 2.90	3.30 0.85	0.77
PLT	800.50 ± 184.56	498.50 ± 191.63	296.00 ± 4.24	412.50 ± 426.39	0.35	601.50 ± 488.61	421.00 ± 287.09	595.50 ± 129.40	816.00 ± 202.23	0.67

Level of significance $p \leq 0.05$

APPENDIX IV

Table 4.4: The mean concentration of haematological parameters between pregnant and non-pregnant Wister rats in relation to their experimental group.

Parameters	Group A			Group B			Group C			Group D		
	NP Wister rats	**P Wister rats**	***p*-value**	**NP Wister rats**	**P Wister rats**	***p*-value**	**NP Wister rats**	**P Wister rats**	***p*-value**	**NP Wister rats**	**P Wister rats**	***p*-value**
WBC	12.59 ± 0.2	5.34 ± 2.52	0.06	12.73 ± 0.05	5.52 ± 2.08	0.39	7.46 ± 1.32	13.15 ± 5.52	0.29	8.24 ± 5.86	20.86 ± 6.80	0.19
NEU	36.8	31.0	0.85	20.1	24.5	0.79	8.15	36.75	0.03	22.75	41.60	0.06

	± 18.95	5 ± 34.2		0 ± 9.76	5 ± 18.3		± 0.35	± 7.43		± 0.78	± 6.51	
BASO	0.25 ± 0.07	2.55 ± 3.18	0.41	0.30 ± 0.14	1.05 ± 0.92	0.37	3.40 ± 0.28	0.35 ± 0.21	0.00 7	0.25 ± 0.21	0.40 ±0.14	0.49
EOS	0.50 ± 0.57	1.10 ± 0.99	0.53	0.35 ± 0.07	2.25 ± 2.76	0.43	0.75 ± 0.64	3.60 ± 3.25	0.35	0.30 ± 0.28	1.80 ± 1.56	0.31
LYM	33.6 58.1	28.1 62.4	0.89	31.4 75.8	33.04 68.9	0.11 0.73	33.63 86.1	38.23 54.45	43.06 0.02	35.60 73.30	0.17 52.90	0.06
MONO	4.45 ± 6.63	2.85 ± 1.65	0.37	3.40 ± 3.34	3.25 ± 3.57	0.92	4.85 ± 2.90	0.28 ± 10.39	3.40 ± 5.74	3.30 ± 0.85	0.94	
PLT	488.61 ± 184.56	601.50 ± 800.50	0.64	498.5 ± 191.6	421.0 ± 287.1	0.78	296.00 ± 4.24	595.50 ± 129.40	0.08	412.50 ± 426.39	816.00 ± 202.23	0.35

Parameters	Non-pregnant Wistar rats					Pregnant Wistar rats					
Group	**A**	**B**	**C**	**D**	**p-value**	**A**	**B**	**C**	**D**	**p-value**
Total protein	58.13 ± 20.21	62.45 ± 33.94	75.85 ± 23.90	68.90 ± 27.13	0.73	86.10 ± 1.13	54.45 ± 34.60	73.30 ± 37.11	52.90 ± 32.16	0.06
Albumin	4.45 ± 6.63	2.85 ± 1.65	3.40 ± 3.57	3.25 ± 3.38	0.92	1.60 ± 1.17	4.85 ± 1.03	3.40 ± 3.74	3.30 ± 0.85	0.94
ALT	0.50 ± 0.24	1.91 ± 3.88	0.14	1.91	0.52	3.63 ± 2.90	3.11 ± 3.23	3.23 ± 1.24	2.49 ± 0.67	

Level of significance $p \leq 0.05$

APPENDIX V

Table 4.5: The mean concentration of Biochemical parameters among pregnant and non-pregnant Wistar rats in relation to their experimental groups.

	± 0.21	± 0.28	± 3.51	± 0.61		0.57	0.30	0.27	0.63	
AST	15.09 ± 1.00	15.36 ± 4.07	15.23 ± 5.30	11.36 ± 4.01	0.71	13.97 ± 0.38	10.91 ± 1.29	13.27 ± 4.73	12.24 ± 4.91	0.83
ALP	89.14 ± 6.62	81.47 ± 5.16	67.16 ± 14.16	64.93 ± 10.54	0.17	84.00 ± 1.02	64.64 ± 1.70	61.45 ± 1.12	73.81 ± 13.95	0.10
T.BIL	67.84 ± 4.50	66.78 ± 6.00	49.02 ± 4.11	64.66 ± 20.99	0.41	63.07 ± 6.75	55.65 ± 5.25	65.19 ± 22.49	80.56 ± 41.22	0.77
D.BIL	21.75 ± 0.21	18.90 ± 5.09	15.75 ± 1.06	15.45 ± 1.06	0.20	21.75 ± 0.21	21.00 ± 3.39	17.70 ± 1.27	16.20 ± 0.42	0.10

Parameters	**Group A**	**Group B**	**Group C**	**Group D**

Level of significance $p \leq 0.05$

APPENDIX VI

Table 4.6: The mean concentration of biochemical parameters between pregnant and non-pregnant Wister rats in relation to their experimental group.

	NP Wister rats	P Wister rats	*p*-value	NP Wister rats	P Wister rats	*p*-value	NP Wister rats	P Wister rats	*p*-value	NP Wister rats	P Wister rats	*p*-value
Total protein	33.63 ± 0.21	33.63 ± 0.21	1.0	28.15 ± 1.14	38.23 ± 3.00	0.05	31.44 ± 1.24	43.06 ± 2.38	0.03	33.04 ± 3.10	35.60 ± 5.69	0.63
Albumin	25.46 ± 6.63	32.16 ± 1.67	0.29	24.68 ± 1.65	34.60 ± 1.03	0.02	27.19 ± 3.34	34.79 ± 10.39	0.43	27.13 ± 3.57	37.11 ± 5.74	0.17
ALT	3.24 ± 0.21	3.76 ± 0.57	0.34	3.88 ± 0.28	3.11 ± 0.30	0.12	5.83 ± 3.51	3.23 ± 0.27	0.53	3.38 ± 0.61	3.49 ± 0.63	0.78
AST	15.09 ± 1.00	13.97 ± 0.38	0.28	15.36 ± 4.07	10.91 ± 1.29	0.28	15.23 ± 5.30	13.27 ± 4.73	0.73	11.36 ± 4.01	12.24 ± 4.91	0.86
ALP	89.14 ± 6.62	84.00 ± 1.02	0.39	81.47 ± 5.16	64.64 ± 1.70	0.05	67.16 ± 14.16	61.45 ± 1.12	0.63	64.93 ± 10.54	73.81 ± 13.95	0.55
T.Bil	67.84 ± 4.50	63.07 ± 6.75	0.49	66.78 ± 6.00	55.65 ± 5.25	0.19	49.02 ± 4.11	65.19 ± 22.49	0.42	64.66 ± 20.99	80.56 ± 41.22	0.68
D.Bil	21.75 ± 0.21	21.75 ± 0.21	1.0	18.90 ± 5.09	21.00 ± 3.39	0.68	15.75 ± 1.06	17.70 ± 1.27	0.24	15.45 ± 1.06	16.20 ± 0.42	0.45

Level of significance $p \leq 0.05$

APPENDIX VII

MINISTRY OF HEALTH

KWARA STATE GOVERNMENT

MOH/KS/EU/777/VOL II/242 30th May, 2025.

Re: Evaluating the Effect of Retinol on Selected Hematological Parameters and Histology of Pregnant Wistar Rat.

Ministry of Health Ethical Research Committee (ERC) Assigned number:

ERC/MOH/2025/05/435

Name of Principal Investigator: **SULEMAN FARIDAT REJOICE**

Address of Investigator: Department of Medical Laboratory Science,
Thomas Adewumi Univeristy,
Oko, Kwara, Nigeria.
Telephone: 08080452495

Date of Approval of Application: **30/05/2025**

NOTICE OF APPROVAL TO CARRY OUT RESEARCH

Sequel to your request and the interest of the State Ministry of Health in Health-related research activities to improve the health of the citizens. I am directed to forward to you the approval of the Ministry of Health to carry out the dissertation as itemized in your protocol. The approval I.D **ERC/MOH/2025/05/435**

2. You are mandated to acknowledge the State Ministry of Health by your presentations/publications and deposition of the final copy of the research findings/publications.

3. Please kindly note that the Ethical Review Committee reserves the right to conduct monitoring/oversight to your research site without prior notification.

4. The Approval dates from 30/05/2025 to 29/05/2026, if there is delay in starting the research, please inform the MOH/ERC so that the date of the approval can be adjusted accordingly.

5. Best wishes in your research project.

Dr Lambe Kudirat Funmi
Chairman Ethical Review Committee
For: Honourable Commissioner

KWARA STATE GOVERNMENT

Original Collected by me
Suleman Faridat Rejoice
July 17 2025

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